Pollinator trait diversity: functional implications at different land-use intensities and environmental conditions

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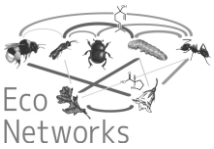

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Zwei Dinge sind zu unserer Arbeit nötig: Unermüdliche Ausdauer und die Bereitschaft, etwas, in das man viel Zeit und Arbeit gesteckt hat, wieder wegzuwerfen.

Albert Einstein

Contents

Summary

It is increasingly noticed that species richness alone is not a sufficient predictor of ecosystem functioning and resilience. The diversity of species responses to environmental variation could be a stabilizing factor to ecosystems, as it can ensure a higher probability that at least some species continue to perform their functions even at unfavorable conditions. Specific functional traits (morphological-, physiological- or life-history traits) could be more beneficial in some habitats and under certain conditions, than others. When species with particular traits and responses to environmental variation are lost, this may cause changes in ecosystem processes and thus have profound consequences for services that humans depend on. It is critical that we understand how these different issues of species diversity influence the role of organisms in ecosystem functioning and resilience. Therefore, we investigated the response diversity and functional traits in pollinator communities experiencing different land-use intensities and varying environmental conditions in three studies.

The first study focuses on the variation of thermal niches of pollinator communities and whether the diversity of thermal responses and the projected thermal resilience are affected by land-use intensity. We recorded pollinator communities including flies, bees, beetles and butterflies (511 species) in 40 grasslands at various weather conditions and determined the thermal niche of each species. Temperature generally explained 84% of the variation in pollinator activity. Species in more intensively used grasslands had broader thermal niches and were also more complementary in their thermal optima. Quantified thermal resilience increased with land-use intensification mainly driven by flies that prefer cooler temperatures and compensated for losses of other taxa. We show that the diversity of thermal responses of pollinators contributes to a higher projected resilience of ecosystems under land-use change.

The second study addressed to the variability in water loss as physiological trait of pollinators that can influence many aspects of species performance. In the view of ongoing climate change, associated with rising temperatures and longer periods of dryness, water loss can be crucial. We measured water loss of 67 pollinator species gravimetrically at extremely dry air conditions for two hours at 15° and 30°C. To investigate differences in water loss of pollinators, we quantified for the first time surface to volume ratios (*SA/V* ratios) of insects, by creating 3D surface models as obtained by structured light scanning methods. Quantified *SA/V* ratios better explained the variation in water loss across species than body mass alone. Small insects with a proportionally larger surface area had the highest water loss rates and, therefore, are most vulnerable to high temperatures and dryness. The four orders did not differ

significantly. Directly measured *SA/V* ratios thus provide a promising method to predict physiological responses of insects, improving the potential of extrapolated relative changes of *SA/V* ratios based on body mass allometry alone.

The third study shows how morphological traits of pollinator communities are filtered by land-use intensity. We recorded pollinator communities on 40 grassland sites along a land-use gradient and measured several morphological characteristics of 476 pollinator species. Community means of body size, hairiness, relative wing size and proboscis length decreased with land-use intensity, although species diversity remain constant. Relative femur length, eye size, antenna length and mandible length were unaffected by land-use. The variation in size of head and relative size of wings and eyes increased with land-use intensity. Shifts in trait means with land-use intensity strongly correspond to shifts in relative abundance of insect orders, whereas consistent land-use changes were rarely found within an order. These findings highlight that functional traits may be more sensible indicators of land-use effects than species diversity alone, and many of these traits can be relevant for ecosystem functionality.

Taken together, quantifying the diversity of responses and mean functional traits of pollinator communities is a promising approach to assess the vulnerability of ecosystems to land-use intensification and climate change. Knowledge about the vulnerability of communities to several factors and the potential for predictions enables us to initiate steps for protection, rather than just documenting the negative impact of already occurred disturbances. And unlike a community of specific species, functional traits can be easily generalized across functional groups and extrapolated to different regions.

Zusammenfassung

Es wird zunehmend festgestellt, dass die Anzahl der Arten eine Ökosystems allein kein ausreichender Prädiktor sein Funktionieren und die Resilienz ist. Die Diversität der Reaktionen von Arten auf Umweltschwankungen (im Folgenden bezeichnet als „response diversity“) könnte ein stabilisierender Faktor für Ökosysteme sein, da sie eine höhere Wahrscheinlichkeit gewährleistet, dass zumindest einige Arten ihrer Funktion nachkommen können, auch wenn die Bedingungen ungünstig sind. Spezifische funktionelle Merkmale (morphologische, physiologische oder lebensgeschichtliche) könnten in bestimmten Lebensräumen und bei speziellen Bedingungen, gegenüber anderen von Vorteil sein. Wenn Arten mit speziellen Merkmalen und Reaktionen auf Umweltveränderungen verloren gehen, kann das Änderungen in Ökosystemprozessen auslösen und so tiefgreifende Konsequenzen für Ökosystemdienstleistungen haben, von denen Menschen abhängig sind. Es ist entscheidend, das wir den Einfluss von diesen verschiedenen Aspekten von Diversität und ihre Rolle für das Funktionieren und die Resilienz von Ökosystemen verstehen. Daher untersuchen wir mithilfe von drei Studien die „response diversity“ und funktionelle Merkmale von Bestäubergemeinschaften, die unterschiedlichen Landnutzungsintensitäten und schwankenden Umweltbedingungen ausgesetzt sind.

Die erste Studie konzentriert sich auf die Variation von Temperaturnischen von Bestäubern und ob die Diversität der Reaktionen auf Temperatur sowie die thermische Resilienz von der Landnutzungsintensität beeinflusst werden. Wir haben Bestäubergemeinschaften inklusive Fliegen, Bienen, Schmetterlinge und Käfer (511 Arten) auf 40 Grünlandflächen bei unterschiedlichen Wetterbedingungen erfasst und die Temperaturnische von jeder Art ermittelt. Die Temperatur erklärte generell 84% der Variation in der Bestäuberaktivität. Arten in intensiver genutztem Grünland haben dabei breitere Temperaturnischen und sind komplementärer in ihrem Temperaturoptimum. Die quantifizierte thermische Resilienz stieg mit der Landnutzung, was hauptsächlich von den Fliegen verursacht wurde, die kühlere Temperaturen bevorzugen und die Verluste der anderen Taxa kompensierten. Dies zeigt, dass die „response diversity“ zu einer voraussichtlich höheren Resilienz von Ökosystemen beitragen kann, die Landnutzungsänderungen unterliegen.

Die zweite Studie befasst sich mit der Variabilität des Wasserverlustes als physiologische Eigenschaft der Bestäuber, der viele Aspekte der Leistungsfähigkeit von Arten beeinflussen kann. Im Rahmen des fortschreitenden Klimawandels, der einhergeht mit steigenden Temperaturen und längeren Trockenperioden, kann hoher Wasserverlust zum Problem werden. Wir haben den Wasserverlust von 67 Bestäuberarten während 2 Stunden bei 15°C und bei 30°C und extrem trockener Luft gravimetrisch gemessen. Um die Unterschiede im Wasserverlust zu untersuchen, haben wir zum ersten Mal

Oberflächen-Volumen Verhältnisse (*A/V*-Verhältnisse) von Insekten quantifiziert. Dafür wurden mit Hilfe der „Structured-Light-Technologie“ 3D-Oberflächenmodelle erstellt. Die berechneten *A/V*-Verhältnisse erklären die Schwankungen im Wasserverlust zwischen den Arten besser, als die Körpermassen allein. Kleine Insekten mit ihrer verhältnismäßig großen Oberfläche wiesen die höchsten Wasserverlustraten auf und sind daher am empfindlichsten gegenüber hohen Temperaturen und Trockenheit. Die vier Insektenordnungen unterschieden sich nicht signifikant. Die direkt gemessenen *A/V*-Verhältnisse stellen eine vielversprechende Methode dar, um physiologische Reaktionen von Insekten vorherzusagen und verbessern die Möglichkeiten der Körpermassen-Allometrie relative Änderungen der *A/V*-Verhältnisse vorherzusagen.

Das Kapitel IV zeigt, dass morphologische Merkmale von Bestäubergemeinschaften von der Landnutzungsintensität gefiltert werden. Wir haben Bestäubergemeinschaften auf 40 Gründlandflächen entlang eines Landnutzungsgradienten erfasst und verschiedene morphologische Merkmale von 476 Bestäuberarten vermessen. Die mittlere Körpergröße, Behaarung, relative Flügelgröße und Rüssellänge der Gemeinschaften sank mit der Landnutzungsintensität, obwohl die Anzahl der Arten konstant blieb. Die relative Größe der Femora, Augen, Antennen und Mandibeln wurden nicht von der Landnutzung beeinflusst. Die Variabilität der Kopfgröße und der relativen Größe von Flügeln und Augen stieg mit der Landnutzung. Die Verschiebung der Merkmals-Mittelwerte mit der Landnutzungsintensität deckte sich stark mit Verschiebungen in der relativen Abundanz der Insektenordnungen, wohingegen innerhalb der Ordnungen kaum Änderungen zu verzeichnen waren. Diese Ergebnisse zeigen auf, dass verschiedene funktionelle Merkmale sinnvollere Indikatoren von Landnutzungseffekten sind, als die Artenzahl allein.

Zusammen genommen ist die Erfassung der „response diversity“ und der Mittelwerte von funktionellen Merkmalen einer Gemeinschaft ein vielversprechender Ansatz um die Sensitivität eines Ökosystem gegenüber der Intensivierung der Landwirtschaft und dem Klimawandel abzuschätzen. Das Wissen über die Sensitivität von Gemeinschaften gegenüber verschiedenen Faktoren sowie das Potenzial Vorhersagen zu machen, ermöglicht uns Schritte zum Schutz einzuleiten, anstatt nur die negativen Einflüsse von bereits aufgetretenen Störungen zu dokumentieren. Und anders als spezifische Artgemeinschaften, können funktionelle Merkmale einfach über verschiedene funktionelle Gruppen hinweg verallgemeinert und in verschiedenen Regionen angewendet werden.

Chapter I

General Introduction

Implications of diversity

The interaction between the climatic change and other ongoing human-induced threats, such as land-use intensification, trigger a global declining of biodiversity (Walther *et al.* 2002; Brook, Sodhi & Bradshaw 2008; Flynn *et al.* 2009; Butchart *et al.* 2010). The biodiversity loss, in turn is revealed as a major driver of ecosystem change (Chapin III *et al.* 2000; Hooper *et al.* 2012). Thus, it is critical that we understand how different issues of diversity influence ecosystem functioning and resilience to environmental change (see Box 1). Altered ecosystem processes and reduced resilience has profound consequences for services that humans derive from ecosystems (Chapin III *et al.* 2000). Knowledge of actual mechanisms through which biodiversity could stabilize ecosystems is still fragmentary up to date (Tilman 1999). One further limitation is that most studies have been conducted in relatively small-scale and highly controlled communities with unknown relevance to real-world ecosystems (Kremen 2005; Srivastava & Vellend 2005).

Based on the general assumption of a positive relationship between biodiversity and ecosystem functioning (Holling 1973; Holling 1996; Tilman, Reich & Knops 2006; Neaeem *et al.* 2009) a guiding principle in ecology is the biological insurance hypothesis. These thesis declares that species diversity within a functional group (functional redundancy) can stabilizes ecosystem functioning by providing an insurance against losses of single species (Naeem & Li 1997; Yachi & Loreau 1999). Species diversity can also stabilize ecosystem functioning by dampening individual species fluctuations with time (portfolio effect, Hooper *et al.* 2005; Thibaut & Connolly 2013). Recent studies indicate that species functional traits and the diversity of responses to environmental variation (see Box 1) rather than species richness alone are meaningful predictors of ecosystem functioning (Elmqvist *et al.* 2003; Hooper *et al.* 2005; Petchey & Gaston 2006).

Response diversity occurs when multiple species contributing to the same function respond differently to environmental variation (Elmqvist *et al.* 2003). Such various responses have been proposed to stabilize ecosystems against environmental change (McNaughton 1977; Leary & Petchey 2009; Winfree & Kremen 2009; Laliberté *et al.* 2010; Romanuk *et al.* 2010). This diversity of functionally redundant species can ensures a higher probability that at least some species continue to perform their functions when conditions are unfavorable.

Interspecific variation in species responses could occur due to different functional traits of species. Such traits could bee morphological traits (e.g. body size, hairiness), physiological traits (water loss, metabolism) or life-history traits (nest location, overwintering state, sociality, diet). These characteristics can determine species mobility, requirements for nesting and food resources, or

physiological tolerance. Traits are hypothesized to be filtered by environmental conditions or habitat characteristics (Southwood 1988; Keddy 1992). If certain traits are promoted, ultimately the composition of the community could change, for example through climate and land-use change (Lavorel & Garnier 2002).

In order to make a prediction on the resilience of ecosystems, we need to know how vulnerable the individual species are and what role they play within the community. This is the only way to assess the consequences of perturbations not only for individual species but also for the entire ecosystem.

Box 1 – Glossary of important terms

Functional trait
A trait is a well defined, measureable characteristic of organisms that is used comparatively across species. Functional traits are traits that strongly affect individual performance (McGill *et al.* 2006).

Community
In ecology, a community is an assemblage or association of populations of two or more different species occupying the same geo-graphical area in a particular time.

Ecosystem functioning
Ecosystem functioning describes the whole system's performance. It is composed of multiple quantitative ecosystem processes including biological, geochemical and physical processes

Functional redundancy
Species of communities that can perform similar roles may, therefore, be substitutable with little impact on ecosystem processes (Lawton & Brown 1993).

Response diversity
Response diversity is defined as the range of reaction norms to environmental change among functionally redundant species contributing to the same ecosystem function (Elmqvist 2003).

Resilience
Ecosystem resilience is defined as the amount of disturbance a system can absorb and still remain within the same state (Holling 1973, 1996). Resilience also encompasses the ability of an ecosystem subject to disturbance and change to reorganize and renew itself (Elmqvist 2003).

Pollinator communities as target systems

Pollination by insects or other animals is essential for a majority of plant species, including crops (Klein *et al.* 2007) and wild plants (Kearns, Inouye & Waser 1998; Ollerton, Winfree & Tarrant 2011). Most wild plant species (80%) are directly dependent on insect pollination for fruit and seed set and many other wild plants were found to be limited by pollination (Ashman *et al.* 2004; Knight *et al.* 2005).

Also crop plants profit from pollination although highest volume crops (e.g. rice and wheat) are wind-pollinated. Apart from wild plants especially fruit crops depend on insect pollination, which makes pollination as ecosystem service necessary for human food worldwide (Klein *et al.* 2007).

Many studies deal exclusively with the honeybee (*Apis mellifera* L.) as important pollinator (e.g. Kremen, Williams & Thorp 2002) but diversity of pollinating insects is much larger. Pollinators comprise bees and other insects, mainly flies, beetles, butterflies and moths (Pellmyr 2002, Fig. 1.1). Outside Europe, vertebrates like bird or bats can also be important pollinators (Pellmyr 2002).

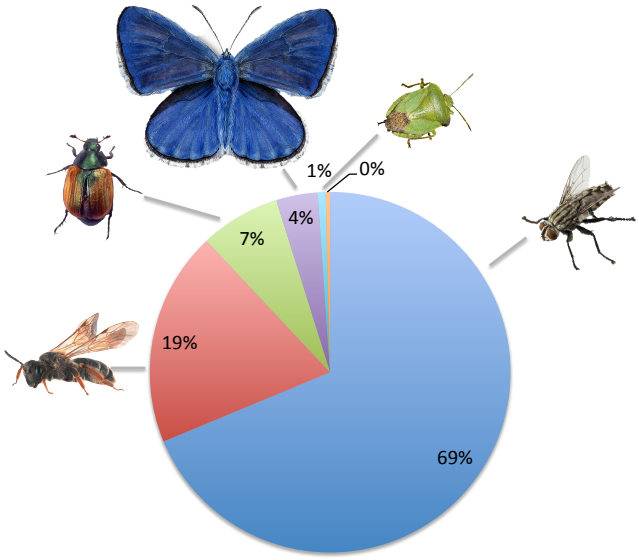

Fig. 1.1: Community of flower visitors on investigated grassland ecosystems.
The percentage of individuals on total species richness (N = 511) is given.

The pollinating insects that visit a plant are oven quite diverse (Herrera 1987; Fishbein & Venable 1996; Sahli & Conner 2007). Different pollinators of a plant species can vary in visitation rates or the effectiveness of pollen transfer (Fishbein & Venable 1996; Vazquez, Morris & Jordano 2005). Furthermore, pollinators vary in their spatial distribution and time of hibernation resulting in different visitation rates to a single plant species (Herrera 1988; Fenster & Diudash 2001; Ivey, Martinez & Wyatt 2003). Bees are often considered to be the most important group of pollinators and their life cycles are closely adapted to plant phenology (Kearns, Inouye & Waser 1998). For many plant communities highly generalist pollinators have a key function, as most plants are not specialized on single pollinator species (Waser *et al.* 1996; Guimarães & Cogni 2002). Although pollinators differ in effectiveness and visitation rates, pollinator importance in several studies was primarily determined by visitation across different pollinator taxa (Vazquez, Morris & Jordano 2005; Sahli & Conner 2007). Thus, frequent pollinator mutualists can contribute the most to pollination, regardless of their effectiveness per visit. But there are also studies highlighting bees as more effective pollinators (Bischoff *et al*. 2013). These bees contributed most to the pollination, even to plants that received just

as many visits by flies. However, especially in in high altitude or latitude systems, or for example in islands where other pollinators are rare, flies are the predominant pollinators (Totland 1993).

Diversity of pollinators recently decline due to multiple drivers (Potts *et al.* 2010). Evidence of pollinator loss comes from studies of pollinator communities along gradients of agricultural intensification, habitat fragmentation, climate change or alien species (Kremen, Williams & Thorp 2002; Winfree & Kremen 2009; Schweiger *et al.* 2010). These local-scale studies suggest a widespread pattern of pollinator loss (Ricketts *et al.* 2008; Winfree & Kremen 2009) as most natural landscapes around the world are anthropogenically modified and, therefore, also concerned by diversity losses.

Since pollinator declines are crucial for the maintenance of pollination as ecosystem service, it is important to investigate effects multiple stressors on diversity, composition and functional performance of pollinator communities.

Land use of grassland ecosystems and its consequences

Most European grasslands developed through anthropogenic farming. Remaining semi-natural grasslands have a particularly high biodiversity, but are changing and disappearing (WallisDeVries, Poschlod & Willems 2002). After the Second World War land-use intensity started to increase and changed grasslands strongly. Application of fertilizer increased, grazing periods get longer, live stock density higher and mowing more frequent (Vickery *et al.* 2001). Chemical fertilizer increases the biomass and allows a higher yield (Tilman *et al.* 2002), but only of few competitive plant species, which affects many herbivores and pollinators (Vickery *et al.* 2001; Kleijn *et al.* 2009). Structure rich, heterogeneous grasslands were partly converted to plant species-poor “bioenergy” producing arable systems (Campbell *et al.* 2008). Fauna impoverished and structurally homogenous rewards developed. Food resources and habitats of species with low mobility were removed by grazing and cutting, which leads to reduced insect species richness and abundance (Vickery *et al.* 2001; Kruess & Tscharntke 2002; Dennis *et al.* 2008). But not all animals are similarly affected by increasing land-use intensity; while many species are disadvantaged, few species seem to benefit (McKinney & Lockwood 1999).

Effects of land-use intensity on pollinators

Increasing land-use intensity is considered to be one of the major drivers of global biodiversity losses and leads to a rising number of threatened species (van Swaay *et al.* 2008; Kleijn *et al.* 2011; Ollerton *et al.* 2014). Floral resources (primarily pollen and nectar) that are reduced in intensively used grasslands are an important determinant of pollinator communities. Bee species richness and abundance is positively related to the species richness and abundance of flowering plants (Banaszak; Steffan-Dewenter & Tscharntke 2001; Potts *et al.* 2003) and species can get lost due to a lack of flowering plant species (Biesmeijer *et al.* 2006; Burkle, Marlin & Knight 2013; Scheper *et al.* 2014; Goulson *et al.* 2015). More specific studies found that bee species richness is affected by the diversity of nectar sources, the ratio of pollen to nectar energy content, and floral morphology (Bosch, Retana & Cerda 1997; Potts *et al.* 2003). Nesting sites are also important determinants of pollinator community composition. Bees utilize a variety of nesting habits, including tunnels in bare ground, pre-existing cavities (e.g. pithy stems) or dead wood (Westrich 1990). Both bee composition and density can be determined by the local presence of potential nesting sites (Eltz *et al.* 2002; Samejima *et al.* 2004; Potts *et al.* 2005). Land-use change alters the distribution of both floral and nesting resources (Tscharntke *et al.* 2005) and thus can affect individual behavior, population dynamics and community composition of bees. Therefore, it is important to find out who is most vulnerable to land use effects and how this affects the community and the stability of ecosystems.

Investigating land-use effects with the “Biodiversity Exploratories”

In order to better understand land-use effects on biodiversity and ecosystem processes the project "Biodiversity Exploratories" was instituted (Fischer *et al.* 2010). In three regions, observations and experiments are conducted on 150 grasslands along a land-use gradient, which allows investigate effects of land-use intensity in different regions.

The regions are: (1) the national park Hainich (UNESCO Natural Heritage) and its surrounding areas in Central Germany with an altitude of 285-550 (annual precipitation 285-550 mm). Apart from the national park the surrounding area is intensively managed, with few remaining extensively managed calcareous grasslands. (2) The UNESCO Biosphere Reserve Schwäbische Alb is located in the Swabian Jura low mountain range in South-western Germany (460-860 m over sea level, annual precipitation 700-1000 mm). This region is very heterogeneous and dominated by grassland and forest patches as well as small arable fields. Some calcareous grasslands are still grazed by traditional sheep herding.

(3) The UNESCO Biosphere Reserve Schorfheide-Chorin is located in the lowlands (3-140 m above sea level) of North-eastern Germany. Mean annual temperatures do not differ greatly between the regions (Schorfheide: 8-8.5°C, Hainich: 6.5–8°C, Alb: 6-7°C).

In each region 150 selected grasslands managed by farmers cover a gradient from very extensive (Fig. 1.2a) to very intensive land-use intensity (Fig. 1.2b). The grasslands are either mown (meadows), grazed (pastures) by different livestock (cattle, sheep, rarely horses) or both (mown pastures). Some grassland sites are treated with a different amount of nitrogen fertilizer.

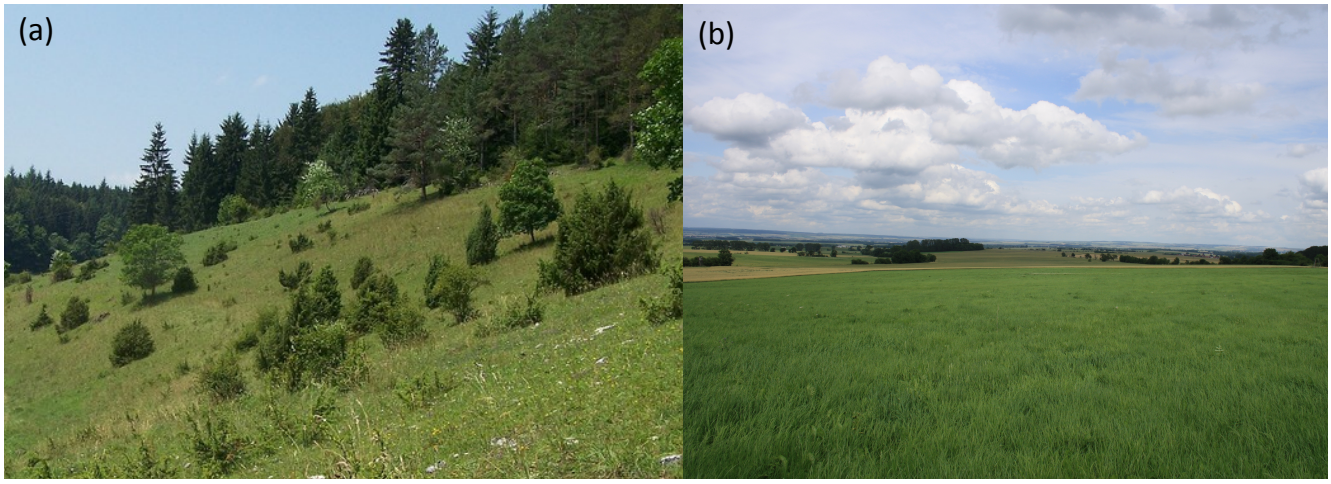

Fig. 1.2: Extensively **(a)** and intensively **(b)** used grassland plots in the "Biodiversity Exploratories".

The land-use intensity is assessed by a land-use intensity index (L_i) (Blüthgen *et al.* 2012). The index combines the amount of fertilizer (F), mowing frequency (M) and grazing intensity (G), based on the information provided by the landowners. The amount of fertilizer F is calculated by the nitrogen input in kg per hectare per year. Mowing M is the number of cuts per year. Grazing intensity G_i represents livestock units differently weighted for sheep, cattle and horse per hectare multiplied by the grazing duration in days per year (livestock units × days × hectar^{-1} × year^{-1}). For each study site i individual components (F_i, M_i, G_i) were calculated and then standardized relative to the global mean of all regions (R).

$$\mathrm{LUI}_i = \frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}$$

Land-use intensity is recorded yearly, but depending on the questions averaged indices for an extended period can be used. I chose 40 grassland sites in the regions Hainich and Schwäbische Alb. The size of the experiment did not allow the exploitation of the whole gradient but we tried to cover a broad land-use gradient with the selected plots.

Climate change

Changes in atmospheric greenhouse gases and aerosols, in solar radiation and in land surface properties alter the energy balance of the climate system (IPPC, 2007). Particularly striking is the tendency of climate warming that express through increases in global air and ocean temperatures, widespread melting of ice, and a rising global sea level. Mean global temperatures have risen this century, and further warming is predicted to continue for the next 50 – 100 years (Parmesan *et al.* 1999). Climate change also involves widespread changes in precipitation amounts, wind patterns and increasing frequency of extreme weather events like droughts, heavy precipitation or heat waves (IPPC, 2007). Global climate change has already altered the conditions of nearly every ecosystem on earth (IPCC, 2007). A key question will be to predict the responses of species and communities to climate change in general, and climate warming in particular. Biological responses are determined by the organism's ecology (Thomas *et al.* 2004; Parmesan 2006), physiology (Deutsch *et al.* 2008; Huey & Pascual 2009), behavior (Kearney, Shine & Porter 2009) and the evolutionary response (Davis, Shaw & Etterson 2005).

Effects of climate change on pollinators

General declines in bee species richness triggered, among others by climate change, are projected for the future (Dormann *et al.* 2008). Pollinators with narrower climatic niches thereby seemed to be more vulnerable to declines (found for British bumblebees, Williams 2007). Impacts of climate change can occur at all organizational levels from the individual level, population genetics and species level shifts to the community level (Potts *et al.* 2010). Climate warming could impact the activity of ectotherms that directly depend on environmental temperatures (Stone & Willmer 1989). Pole ward shifts in geographical distribution ranges of butterflies are associated with climate warming as well as local or regional extinctions of butterfly species (Parmesan *et al.* 1999). The emergence of dispersive phenotypes will increase the speed at which species ranges shift and new environments are established (Thomas *et al.* 2001).

Species do not necessarily react similarly to global change, which bears the risk of disrupting plant – pollinator interactions. Climate change-induced mismatches could be temporal or spatial. Generally, the begin of blooming of plants and the first appearance of pollinators in the year appear to advance similar in response to recent temperature increases, but other studies found temporal mismatches among pollinator-plant interactions (Bartomeus *et al.* 2011; CaraDonna, Iler & Inouye 2014).

Simulations indicate that shifts in phenology (e.g. earlier appearance in spring) have the potential to disrupt the temporal overlap between pollinators and their floral food resources (Memmott *et al.* 2007, but see Blüthgen 2010). Ecological-niche models for a monophagous butterfly and its larval host plant predict that climate change can result in a pronounced spatial mismatch between future niche spaces of these species (Schweiger *et al.* 2008). The butterfly may expand its future range if the host plant has unlimited dispersal, but range could be reduced if the host plant is limited in dispersal. But rising temperatures may not only have effects at the long-term scale, but also a direct influence on species behavior in terms of changing activities. If under unfavorable conditions, the activity of some pollinator declines, the ecosystem needs others that can compensate these losses. Otherwise, the maintenance of pollination services and the resilience of ecosystems are at risk.

To date, most studies focused on the influence of increasing temperatures on species, do not consider that also patterns of rainfall and water availability have been changing and are expected to continue doing so. In consequence, water balance of insects becomes more important. Pollinators that suffer high water loss are probably more vulnerable to ongoing climate change. Therefore, we need to know if there are differences in water loss and discover potential reasons. Future changes, which are predicted to increase in their extent, are likely to have even more severe impacts than recent historical changes (Settele *et al.* 2008).

Study objectives

It is increasingly recognized that species richness alone is not the only meaningful predictor of ecosystem functioning at varying land use and environmental conditions (Hooper *et al.* 2005; Petchey & Gaston 2006). It is critical that we understand how different issues of species diversity influence the role of organisms in ecosystems, potential impacts of community changes and species losses and particularly ecosystem resilience. To achieve this, we want to investigate the role of functional traits and response diversity in pollinator communities that experience different land-use intensities and at variable environmental conditions.

Chapter outline

Chapter II

Climate warming and intensification of land use are generally expected to be a threat for the maintenance of ecosystem resilience. But it is still poorly understood whether variation in temperature responses between species in a community represents a stabilizing element to fluctuating conditions. We recorded thermal niches of pollinator species and extrapolate variation in thermal niches between species to the community level. Furthermore, we studied the changes in thermal niches of pollinator communities and projected thermal resilience with land-use intensity. Therefore, we recorded 14873 individuals from 511 pollinator species on 40 experimental grassland sites along a land-use gradient during different temperature conditions.

Chapter III

Despite the importance to understand future responses to a warming climate, it is largely unknown which species are most vulnerable to change. Temperature-dependent performance traits such as water loss provide the opportunity to enhance the understanding of variation in responses. We investigated whether various pollinators differ in their relative water loss at different temperatures and whether these differences correspond to taxonomic groups, water content, body mass and *SA/V* ratio. Hence, we studied water loss of 67 pollinator species at different temperatures and developed a method to create 3D-models of insects with a structured light scanner to explain differences in water loss.

Chapter VI

One of the challenges in ecology is to figure out whether species with certain functional traits will persist in habitats under land use. This requires an understanding of the relationships between habitat characteristics and functional traits of species that occur in the habitats. The central question was weather land-use intensity filters specific functional traits and thus determines which species can occur in which habitats. Hence, we measured morphological traits of over 450 pollinator species from 40 grassland sites and related them to land-use intensity.

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Declaration of Authorship

Chapter II

„High diversity stabilizes the thermal resilience of pollinator communities in intensively managed grasslands“

Sara Kühsel, Nico Blüthgen

N.B. designed the study. S.K. collected field data. Both authors performed modeling work and analyses. S.K. drafted the manuscript, and both authors contributed substantially to writing.

Chapter III

„Surface area - volume ratios in pollinating insects“

Sara Kühsel, Adrian K. Brückner, Sebastian Schmelzle, Michael Heethoff, Nico Blüthgen

N.B. and S.K. conceived water loss measurements. M.H., S.S. and A.K.B. developed 3D-imaging method. S.K. performed water loss measurements. S.K. and A.K.B. conducted 3D-imaging of insects. S.K. and N.B. analyzed the data. SK wrote the first draft of the manuscript and all authors contributed to writing.

Chapter VI

“Land-use intensity as a filter of morphological trait composition: pollinator communities in meadows and pastures”

Sara Kühsel, Nico Blüthgen

N.B. and S.K. conceived trait measurements and analyzed the data. S.K. drafted the manuscript and N.B. contributed to writing.

CHAPTER II

High diversity stabilizes the thermal resilience of pollinator communities in intensively managed grasslands

Sara Kühsel, Nico Blüthgen

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Abstract

The resilience of ecosystems depends on the diversity of species and their specific responses to environmental variation. Here we show that the diversity of climatic responses across species contributes to a higher projected resilience of species-rich pollinator communities in real-world ecosystems under land-use change. We determined the thermal niche of 511 pollinator species (flies, bees, beetles and butterflies) in 40 grasslands. Species in intensively used grasslands have broader thermal niches and are also more complementary in their thermal optima. The observed increase in thermal resilience with land-use intensification is mainly driven by the dominant flies that prefer cooler temperatures and compensate for losses of other taxa. Temperature explained 84% of the variation in pollinator activity across species and sites. Given the key role of temperature, quantifying the diversity of thermal responses within functional groups is a promising approach to assess the vulnerability of ecosystems to land-use intensification and climate change.

Introduction

Ecosystem resilience is a pivotal concept in different contexts ranging from the production of natural resources and other ecosystem services to the conservation of species and natural systems (Naeem & Li 1997; Loreau *et al.* 2001; Elmqvist *et al.* 2003). Species diversity within a functional group (functional redundancy) often stabilizes ecosystem functioning by providing insurance against losses of single species (insurance hypothesis), or by dampening individual species fluctuations (portfolio effect, Hooper *et al.* 2005; Thibaut & Connolly 2013). In simple words, when a species is lost, functionally redundant (or 'equivalent') species may replace its performance temporarily or in the long term. Indeed, for a number of ecosystem services, positive relationships between diversity and stability have been confirmed (Tilman, Reich & Knops 2006). In theory, stability arises from the extent to which 'redundant' species differ in multiple other niche dimensions (Rosenfeld 2002), particularly their responses to environmental conditions (McNaughton 1977; Tilman 1999). Such a response diversity (Elmqvist *et al.* 2003) of functionally redundant species ensures a higher probability that at least some species continue to perform their functions – stabilizing an ecosystem over time when conditions vary (McNaughton 1977; Tilman 1999; Blüthgen & Klein 2011; Brittain, Kremen & Klein 2013).

Whereas the stabilizing role of response diversity is intriguing theoretically, quantitative models are scant, focal environmental conditions are rarely specified, and empirical data from real ecosystems are largely missing (Mori, Furukawa & Sasaki 2013). Instead of using morphological or life-history traits

(Laliberté *et al.* 2010), response diversity may also be defined for target environmental variables. For instance, Cariveau *et al.* (2013) assessed the linear abundance – land use relationship for different species of bees and then defined the variation of slopes across co-occurring species as a measure of response diversity. Their response diversity did not translate into a consistent stabilization of crop pollination services. However, in such concepts based on monotonous or linear environmental responses, stability may not necessarily increase with the diversity of slopes of land-use responses, but by positive slopes per se (i.e. species' tolerances of land use).

In addition to land-use responses (Cariveau *et al.* 2013) or vulnerability to disturbance (Mori, Furukawa & Sasaki 2013) we can also quantify response diversity based on well-defined environmental niche dimensions such as climatic conditions (McNaughton 1977; Tilman 1999). In the present study, we focus on thermal niches of pollinators to characterize response diversity and resilience. Responses to temperature are relevant for the behavior, phenology and distributional ranges for animals, particularly ectotherms, and their ecosystem functioning such as pollination (Hegland *et al.* 2009; Fründ *et al.* 2013). Thermal niches are typically uni-modal (Deutsch et al. 2008; Kingsolver & Huey 2008). Species differ in their thermal tolerance (niche breadth) as well as in their optima (niche complementarity) (Fig. 2.1). The ‘community niche’ is composed of individual species niches that co-occur at the same site. Here we explicitly define the integral defined by the community niche of all co-occurring species that perform a particular function – pollinators – as a proxy of the functional resilience of an ecosystem. Ecological resilience is traditionally defined as the ability of an ecosystem to absorb environmental changes (Holling 1973; Standish *et al.* 2014); here specifically the ability to maintain a functional performance level such as pollinator visitation against variation in climatic conditions. Consequently, broader tolerances of individual species' performances (niche breadth) and the extent of variation across species (niche complementarity) both contribute positively to functional resilience. Figure 2.1 visualizes our general concept and definition of response diversity and resilience for thermal responses that can also be generalized to other environmental variables and uni-modal reaction norms (McNaughton 1977; Tilman 1999). Whereas resilience in a strict sense involves measuring thresholds for ecosystem transitions that are rarely applicable to real communities (Standish *et al.* 2014), our mathematical framework provides a practicable, explicit quantitative prediction for resilience that goes beyond a mere characterization of ‘functional diversity’.

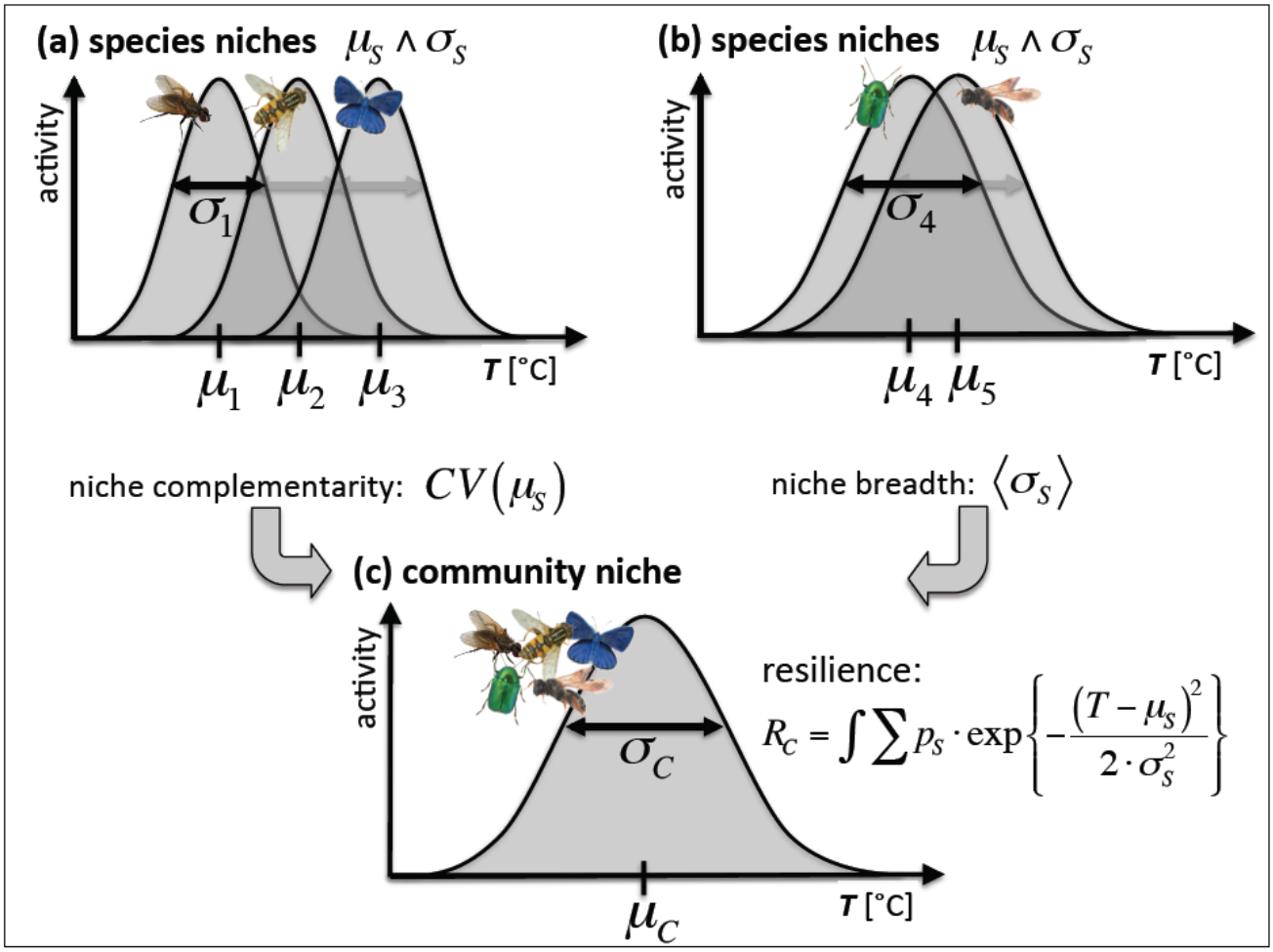

Fig. 2.1: Conceptual framework of community (thermal) niche, response diversity and resilience. A broader community niche and higher thermal resilience may be driven by (**a**) high niche complementarity of species, i.e. strong variation in species mean niche positions (μ_S) and thus *response diversity*, and/or by (**b**) broader species niches (σ_S). The community resilience (R_C) is based on the weighted sum of the individual species niches (**c**), with species weighted by their proportional abundance (p_S).

Different processes are crucial for the maintenance of ecosystems, among which the biomass productivity of plants is the best understood ecosystem function in the context of biodiversity experiments (Hector *et al.* 1999). Pollination by animals is important for three-quarters of the major crop plants (Klein *et al.* 2007) and for the reproduction of an even higher proportion of wild plants (Ollerton, Winfree & Tarrant 2011). Numerous taxa visit flowers and are potential pollinators (Kevan 1983) such as bees, flies, butterflies and beetles. Nevertheless there are concerns regarding the maintenance of pollination services because of recent large-scale declines in pollinator diversity (Biesmeijer *et al.* 2006; Potts *et al.* 2010), which is largely caused by intensive land use (Kremen, Williams & Thorp 2002; Morandin & Winston 2006; Klein *et al.* 2007). High fertilizer application and frequent mowing or grazing may lead to impoverished grasslands with structurally homogenous rewards (Tallowin *et al.* 2005). Grazing and cutting remove floral food recourses and accordingly affect pollinators (Kruess & Tscharntke 2002). However, in the same grasslands as examined in the

present study, Weiner *et al.* (2014) found that total pollinator diversity generally remained at a high level, irrespective of land-use intensity. Whereas diversity was constant, the composition of the pollinator community changed considerably. In intensively used grasslands flies became more dominant whereas butterflies and bees were less frequent. A higher dominance of a taxon, a case of homogenization, likely correspond to more similar response traits and could lead to decreasing response diversity such as a smaller thermal activity range of pollinators.

Little is known about the variance in climatic responses between species in a community, and how this interspecific variation represents a stabilizing element to fluctuating conditions. We aim to extrapolate variation in thermal niches between species to the community level, and to study whether the diversity of thermal responses and the projected thermal resilience of pollinator communities are affected by land-use intensity. First, we quantified the importance of temperature in predicting the general activity of pollinators. Second, we studied the changes in thermal niches of pollinator communities with land-use intensity and whether the projected resilience corresponds to pollinator diversity. Third, we examined how thermal niches vary across different pollinator taxa and with body size. To answer these questions, we investigated pollinator communities on 40 experimental grassland sites along a land-use gradient during different daily and seasonal temperature conditions. Our results show that thermal resilience of pollinator communities increases with land-use intensity, an effect mainly driven by flies that become more dominant. Some species thus maintain ecosystem functions at unfavorable conditions – a consequence of the high level of diversity that occurs along the land-use gradient.

Material and Methods

Study sites

Data were collected between May and September 2012 on grassland plots in the Hainich-Dün region in central Germany (within a radius of 35 km) and in the Schwäbische Alb in southwestern Germany (within a radius of 20 km, see maps in supplementary Fig. 2.4). The plots are part of the Biodiversity Exploratories project (Fischer *et al.* 2010). In our study, 40 plots were selected along a land-use gradient from semi-natural to intensively managed grasslands. Land use can be characterized for each plot by a compound land-use intensity index (LUI, Blüthgen *et al.* 2012) that integrates intensity of fertilization, mowing frequency and grazing intensity. For our analyses we use an averaged LUI from 2011 and 2012 to consider land-use management during the observation year and the previous year, as both may have a direct influence on pollinating insects with a predominantly annual life cycle. To

test for potential long-term effects of land use, we repeated the analysis using a LUI that was averaged from 2006 (when plots were established) to 2012. General results were very similar (see supplementary Table 2.4). To test whether effects are reproducible, we also repeated the analysis for an earlier pollinator survey in 2008 (Weiner *et al.* 2014) from which the records from the Schwäbische Alb and Hainich were used. The majority of plots (49 out of 70) in this earlier dataset were represented by a single day (6 hours) and thus only provide a seasonal snapshot of the community, but collecting methods and transects were the same (Weiner *et al.* 2014).

From the continuous records of climatic conditions by environmental monitoring units, we calculated the mean temperature for our focal plots from May 1st to August 31st 2012 at 10 cm above the ground, a zone with high temperature variation (Geiger, Aron & Todhunter 2009). Mean air temperatures during these summer months ranged from 15.1 to 18.2°C across the plots in the Hainich and from 16.6 to 18.2°C in the Schwäbische Alb.

Plant – pollinator interactions

We surveyed 149 plant-flower visitor interaction networks on 16 plots in the Alb and 24 plots in the Hainich. Size of grassland plots varied between 187.1 and 1.4 ha (mean: 28.4). We observed the plots repeatedly between one (four cases) and 13 times (median: 4 observation days per plot, corresponding to a total observation time mean of 24 h per plot) (see supplementary Table 2.5). Each time a transect of about 300 m^2 per plot was observed for six hours between 08.00 and 14.00 (methods comparable with Weiner *et al*. 2014). The transect was divided into eight sectors of 25 m length and three meter width. Each sector was observed for 15 min, three times a day. During these transects walks all flower visitors were collected. Only insects that touched reproductive parts of the flowers were considered. All animals that could not be identified visually in the field were sampled and identified to species level by taxonomists. All visitors in this analysis are known to pollinate flowers in general (but not necessarily all herbs in these grasslands). Non-pollinating taxa (e.g. bugs) were excluded; Thysanoptera and pollen beetles (*Meligethes sp*.) were not counted; they are mostly hidden within flowers, hence their abundance and activity could not be reliably quantified across different plants.

To assess the importance of flower resources on the plots we counted floral units for all flowering plant species (excl. grasses) or estimated their number by extrapolation from a small area for highly abundant plants. Floral units were defined as one flower or more flowers (e.g. Asteraceae) when flying would be necessary for pollinators to switch between flowers. For each plant species a characteristic

flower was measured. In actinomorphic flowers, flowering area was calculated as a circle based on the flower diameter, whereas the flowering area of zygomorphic flowers was calculated as a rectangle based on flower length and width. Flower cover per species was calculated by multiplying the number of flowering units of a species by its flowering area. Results of each plant species were summed up to calculate the flower cover per plot (see supplementary data 2.1). For reasons of time no new flower cover estimation was done when there were less than four days between the pollinator surveys.

Pollinator species traits

Pollinator responses to temperature and body size as species traits were measured to investigate the role of different pollinators in managed grasslands. Air temperature was recorded at 15 min intervals at the height of the vegetation with the portable weather station TFA Nexus 35.1075 (TFA Dostmann GmbH & Co. KG, Germany). For each pollinator individual the actual temperature at which it was observed was assigned. Plots were repeatedly visited at different weather conditions, but even observations were not possible due to mowing and grazing. Across all sites we observed pollinators at air temperatures between 5 and 37°C.

We measured body size of each one individual per species using a stereomicroscope with digital camera and software after calibration (Motic® SMZ 168). Head, thorax and abdomen were measured separately before total body length was calculated as the sum, avoiding problems of variation in abdomen position etc. Body lengths for unavailable specimen were supplemented with values from the literature in 6 cases. All species names, body size and predicted body mass are listed in the supplementary data 2.2. We converted body length (mm) of pollinator species to dry body mass (mg) using average body mass estimates of conversion equations for each insect order provided by Dillon and Frazier (2013). This method is recommended for community analyses (Saint-Germain *et al.* 2007) since biomass is a better indicator of the functionality of a species within a community than its size.

Thermal niche

Temperature-specific pollinator activity (A_T) within intervals of 15 min was calculated by the sum of individuals per temperature T divided by the number of intervals in which this temperature was recorded. This standardization accounts for differences in the frequency at which temperatures were recorded. To characterize the thermal niche of each pollinator species we supplemented the data from 2012 by pollinator activities recorded together with air temperatures in an earlier study (Weiner *et al.*

2014) conducted in 2008, yielding activity data from a total of 35875 pollinator individuals. The high number of individuals enabled us a detailed analysis of the temperature niches also of rare species. To characterize the pollinator community on a plot, all species and their number of individuals were pooled over the whole season in our target year 2012.

Most environmental niches of species are represented by unimodal functions, typically Gaussian curves of the activity or functional performance. Here, the unimodal thermal niche of each species S is characterized by two parameters: the weighted mean μ_S temperature (T) at which it was recorded, and the weighted standard deviation σ_S (Fig. 2.1). The mean μ_S represents the species' thermal optimum, σ_S its niche breadth. Therefore,

$$\mu_S = \sum_{T=5°C}^{40°C} w_{S,T} \cdot T \text{ and } \sigma_S = \sqrt{\sum_{T=5°C}^{40°C} w_{S,T} \cdot \left(T - \mu_S\right)^2},$$

where $w_{S,T}$ is the proportional weight (sum of weights = 1 for each species S). Weighting is based on both the activity rate $A_{S,T}$ (standardized number of individuals per 15 min per T) and the sample size $N_{S,T}$ (number of individuals per T) as

$$w_{S,T} = \frac{A_{S,T} \cdot N_{S,T}}{\sum_T A_{S,T} \cdot N_S},$$

where N_S is the total number of individuals of the species S. This weighted approach considers the relative temperature preferences (rates) as well as the reliability (number of observations per temperature) to characterize a species' niche. To test the effects of individual weighting on the niche characterization, we also calculated μ_S with weights $w_{S,T}$ based on activity rates $A_{S,T}$ and on number of individuals $N_{S,T}$ alone. Niche characterization was robust against the choices of weights. Mean μ_S based on these alternative weights are highly correlated to our preferred combined weighting (Spearman rank, $r = 0.96$, $p < 0.001$ for $A_{S,T}$; $r = 0.92$, $p < 0.001$ for $N_{S,T}$, $n = 511$ species).

Each community (defined as a set of co-occurring species at a site) can be summarized by three parameters: the average species' optima, their variation across the species that defines their complementarity (i.e. response diversity), and the mean niche breadths of the species. All measures are weighted by the proportional abundance of each species in the community p_S. The weighted mean niche optimum (Fig. 2.1) across the species-specific μ_S in the community is defined as

$$\mu_C = \sum_{S=1}^{\max(S)} p_S \cdot \mu_S ,$$

the weighted coefficient of variation (CV) in μ_S to represent the niche complementarity across species as

$$CV_C = \frac{\sigma_\mu}{\mu_C} , \text{ with } \sigma_\mu = \sum_{S=1}^{\max(S)} p_S \cdot \left(\mu_S - \mu_C\right)^2 ,$$

and the weighted mean niche breadth (σ_S) as

$$\left\langle \sigma_S \right\rangle = \sum_{S=1}^{\max(S)} p_S \cdot \sigma_S .$$

Generally, stabilizing effects of species diversity are suggested to be strongly influenced by abundance (Hector *et al.* 2010). The extinction of a single abundant species can lead to a high impairment of ecosystem functioning if this species strongly contributes to the target process (Hillebrand, Bennett & Cadotte 2008). Therefore we include species relative abundances (p_S) in the analysis of thermal niches here. Weighting species by abundance also accounts for potential inaccuracies of thermal niches of species with few observations. Nevertheless, to examine a potential bias by rarely observed species, we repeated the linear mixed effect models after excluding 291 species with fewer than 5 individuals. Effects of land-use intensity on thermal niches of pollinators remained largely unchanged for this reduced dataset, supporting the robustness of the weighted analysis (see supplementary Table 2.6).

While weighting of the niche parameter by species abundances may better characterize communities, it does not consider possible compensatory dynamics. For instance, frequent species may become rare, and in turn rare species may become more frequent due to competitive release. Hence we additionally calculated thermal niches for unweighted niche parameters and obtained similar results (see supplementary Table 2.7).

As the community curve may be multimodal if variation in μ_S is large compared to σ_S, the weighted standard deviation of the community performance (σ_C in Fig. 2.1) does not fully characterize its resilience. Instead, the thermal resilience of the community R_C is defined as the integral of the summed species curves, hence

$$R_C = \int_{T=5°C}^{40°C} \sum_{S=1}^{\max(S)} p_S \cdot \exp\left\{-\frac{(T-\mu_S)^2}{2\cdot\sigma_S^2}\right\},$$

which is again weighted by p_S, being the relative abundance of species S.

To facilitate comparisons of different communities that differ in the amplitude of the activity curves, R_C is standardized by dividing it by the maximum amplitude; this normalizes all communities to the same maximum of 1.

Note that this concept of thermal niches and resilience, as any trait dimension used in the context of response diversity so far, is based on fixed trait values per species – neglecting the potential of inter-population variability and individual plasticity (Mori, Furukawa & Sasaki 2013). The resulting estimations of resilience may thus be considered conservative, but this should not bias the relative differences between communities, i.e. the main scope of our study. Conceptually, resilience can increase by two drivers: higher niche breadth and higher niche complementarity (Fig. 2.1). Niche complementarity and breadth are independent, since our definition of complementarity only focuses on variation in thermal optima rather than on niche overlap.

Data analyses

Statistics were conducted in R 2.15.1 (R Development Core Team, 2012). We fitted a Gaussian function using the "nls" algorithm in R to describe the relationship between total pollinator activity and temperature. To estimate the goodness-of-fit, we derived an r^2 from a Pearson's correlation between observed and fitted values. The effects of land-use intensity on mean pollinator diversity and plant diversity per plot as well as the influence of flower cover on mean pollinator abundance per plot were assessed with linear models. We used the exponential form of Shannon diversity $e^{H'}$ as measure for diversity to consider different abundances of species. To estimate the effects of land-use intensity on the abundance of different pollinators, we divided the pollinators in different taxonomical groups (in analogy to Weiner *et al.* 2014) and analyzed them separately with linear models: bees (67 species), beetles (49), butterflies (28), other flies (276), other hymenopterans (18) and hoverflies (73). Hoverflies and bees were separated from flies and hymenopterans, respectively, as they are commonly used as bioindicator taxa (Biesmeijer *et al.* 2006). We transformed by arcsine square root the proportion of pollinator taxa in the community and the flower cover to meet the assumptions of homoscedasticity.

To estimate the main and interaction effects of LUI and region (Exploratory) and the main effect of species diversity on thermal optima, thermal niche breadth, thermal niche complementarity and community niche area, we fitted four linear mixed models. The mean air temperature during the observations on each plot was employed as random factor to control for a potential bias of conditions in each plot, but the general results remained unchanged when this random factor was removed. We ran analyses for all taxa, for flies (the dominant taxon), and all taxa excluding flies to identify whether flies are responsible for land-use effects.

Results

General findings

In total 14873 pollinator individuals from 511 species and 64 families belonging to the orders Diptera (64 %), Hymenoptera (28 %), Coleoptera (5 %) and Lepidoptera (3 %) were collected from 40 plots. A total of 143 species of plants were flowering during our surveys, of which 104 were visited by pollinators. The species diversity of both flowering plants and pollinators was high: the mean effective diversity ($e^{H'}$) per plot per day was 4.43 ± 1.82 for flowers and 26.05 ± 10.86 for pollinators. Land-use intensity (LUI) had a negative impact on flower diversity (linear model (lm), LUI: $F_{1,36}$ = 5.5, p = 0.024, region: $F_{1,36}$ = 4.5, p = 0.041; significant in the Schwäbische Alb but not in the Hainich. In contrast, pollinator diversity did not change with land-use intensity (lm, LUI: $F_{1,36}$ = 0.1, p = 0.81, region: $F_{1,36}$ = 3.8, p = 0.056).

The composition of the plant community changed significantly with land-use intensity. The proportion of Asteraceae on total flower cover per plot increased from about 10% on extensive plots to over 50% on intensively used plots, whereas the proportion of Fabaceae decreased from 40% to 10%. The proportion of other abundant plant families did not change significantly (see supplementary Table 2.1, Fig. 2.1). We also found changes in the composition of the pollinator community with land-use intensity. The proportion of flies increased significantly with land-use intensity (lm, $F_{1,38}$ = 5.1, p = 0.029) except hoverflies that showed the opposite trend ($F_{1,37}$ = 3.3, p = 0.079). The proportion of butterflies decreased with LUI ($F_{1,26}$ = 9.9, p = 0.004), as well as the bees in the Schwäbische Alb ($F_{1,14}$= 13.3, p = 0.003), but not in the Hainich ($F_{1,22}$= 0.2, p = 0.70). There was no consistent change in the proportion of other hymenopterans and beetles.

Thermal niche

The activity of pollinators was highly correlated with air temperature, as expected for ectotherms. Some 84 % of the variation in total pollinator activity was explained by the temperature, closely following a Gaussian function (Fig. 2.2a). In contrast, flower cover on the plots during the surveys had no consistent effect on pollinator abundance (lm, $F_{1,37} = 2.4$, $p = 0.13$), confirming the primary importance of temperature for pollinator activities.

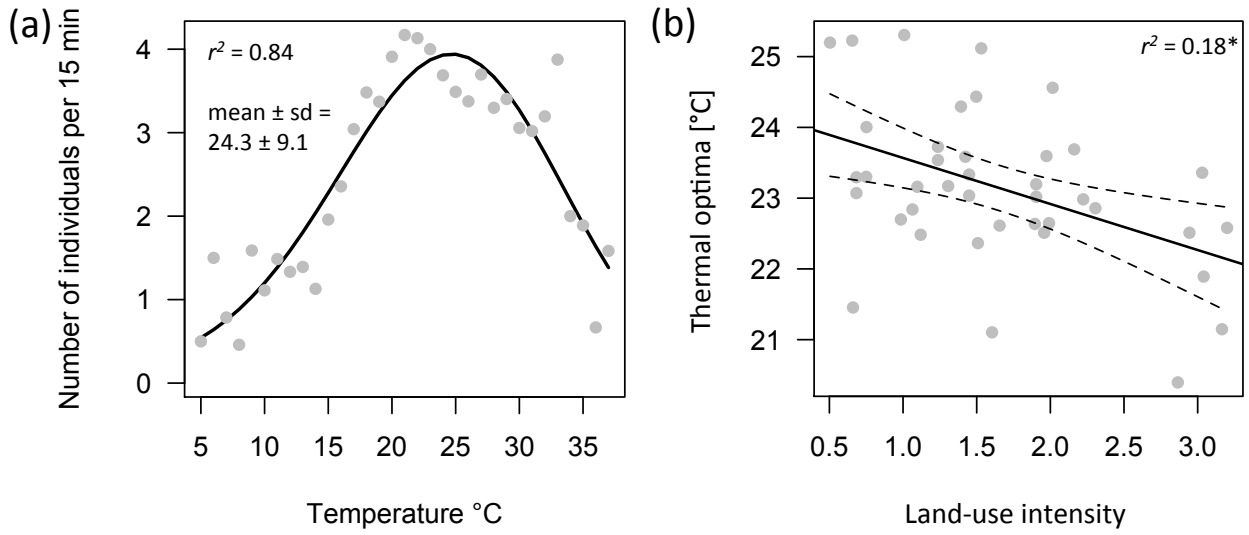

Fig. 2.2: Temperature preferences of pollinators. Effects of temperature on total pollinator activity (**a**), where each data point corresponds to the mean number of individuals recorded per 15 min during a given temperature (all surveys in 2012 pooled). (**b**) Community thermal optima (weighted means across all species occurring in a plot) in response to land-use intensity. Significances: *p < 0.05, **p < 0.01, ***p < 0.001. Dotted lines represent 95% confidence intervals.

To investigate differences in the thermal niches of pollinators, we calculated for all 511 species a thermal optimum (μ_S) and for 406 species (all with N > 1) a thermal niche breadth (σ_S). The mean thermal optimum of a community (μ_C) decreased with land-use intensity (Table 2.1, Fig, 2.2b), indicating that pollinator species in intensively used grasslands preferred colder temperatures than those on extensive grasslands. Thermal optima μ_S varied across pollinator taxa (Fig. 2.4b). Bees and butterflies preferred warmer temperatures than other hymenopterans, flies and beetles.

Table 2.1: Determinants of thermal niches of pollinator communities. Effects of pollinator taxa, land-use intensity (LUI, average of 2011 and 2012), Exploratory (region) and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Results are from linear mixed models.

All taxa					**Flies only**				
	df_{num}	df_{den}	F	p		df_{num}	df_{den}	F	p
Thermal optima					**Thermal optima**				
Taxa	5	168	16.4	**< 0.001**					
LUI	1	36	6.8	**0.013**	LUI	1	35	21.3	**< 0.001**
Region	1	36	2.8	0.105	Region	1	35	8.6	**0.006**
LUI × Region	1	36	0.2	0.638	LUI × Region	1	35	3.3	0.077
Diversity	1	168	0.5	0.486	Diversity	1	35	2.1	0.159
Thermal niche breadth					**Thermal niche breadth**				
Taxa	5	141	14.5	**< 0.001**					
LUI	1	36	11	**0.002**	LUI	1	35	3.7	0.063
Region	1	36	1	0.315	Region	1	35	2.2	0.147
LUI × Region	1	36	0.2	0.64	LUI × Region	1	35	0.1	0.773
Diversity	1	141	0.8	0.388	Diversity	1	35	4.6	0.038
Thermal niche complementarity					**Thermal niche complementarity**				
Taxa	5	141	18	**< 0.001**					
LUI	1	36	15	**< 0.001**	LUI	1	35	11.1	**0.002**
Region	1	36	0.6	0.444	Region	1	35	0.7	0.416
LUI × Region	1	36	0.8	0.374	LUI × Region	1	35	0.2	0.687
Diversity	1	141	1.1	0.304	Diversity	1	35	2.2	0.146
Community niche area					**Community niche area**				
LUI	1	35	13.8	**< 0.001**	LUI	1	35	4.7	**0.037**
Region	1	35	0.1	0.775	Region	1	35	2	0.171
LUI × Region	1	35	0.9	0342	LUI × Region	1	35	0.8	0.227
Diversity	1	35	0.3	0.611	Diversity	1	35	1.5	0.366

Average species niche breadths $\langle \sigma_S \rangle$ (Fig. 2.3a, Table 2.1) as well as niche complementarity CV_C (Fig. 2.3b, Table 2.1) significantly increased with land-use intensity. Thermal generalists were thus more common in intensively used grasslands, and co-existing species in such grasslands differed to a greater extent in their thermal optima. Both effects were independent of pollinator diversity (Table 2.1). In consequence, community niche area (R_C) as an appropriate proxy measure for resilience, increased with land-use intensity (Fig. 2.3c), and the effect was consistent in both regions (Table 2.1). The product of $\langle \sigma_S \rangle$ and CV_C strongly predicted the variation in R_C (lm, $F_{1,38} = 16.8$, $p < 0.001$, Fig. 2.3c). The resilience R_C considers variability along the entire temperature range (5°C – 40°C), thus we additionally examined how these communities may respond to warmer conditions (35°C – 40°C) that become increasingly common with global warming. Despite the negative trend in μ_S, a high level of

resilience R_C for warm conditions was maintained across the land-use intensity gradient (linear mixed model (lme), $F_{1,35} = 3.8$, $p = 0.060$; with a marginally significant increase that was consistent across regions, $F_{1,35} = 0.5$, $p = 0.18$). For pollinator data from 2008, recorded on 70 grassland plots in the same regions, we confirmed a marginally significant trend that niche breadth, complementarity and community niche area increased with land-use intensity (see supplementary Table 2.2).

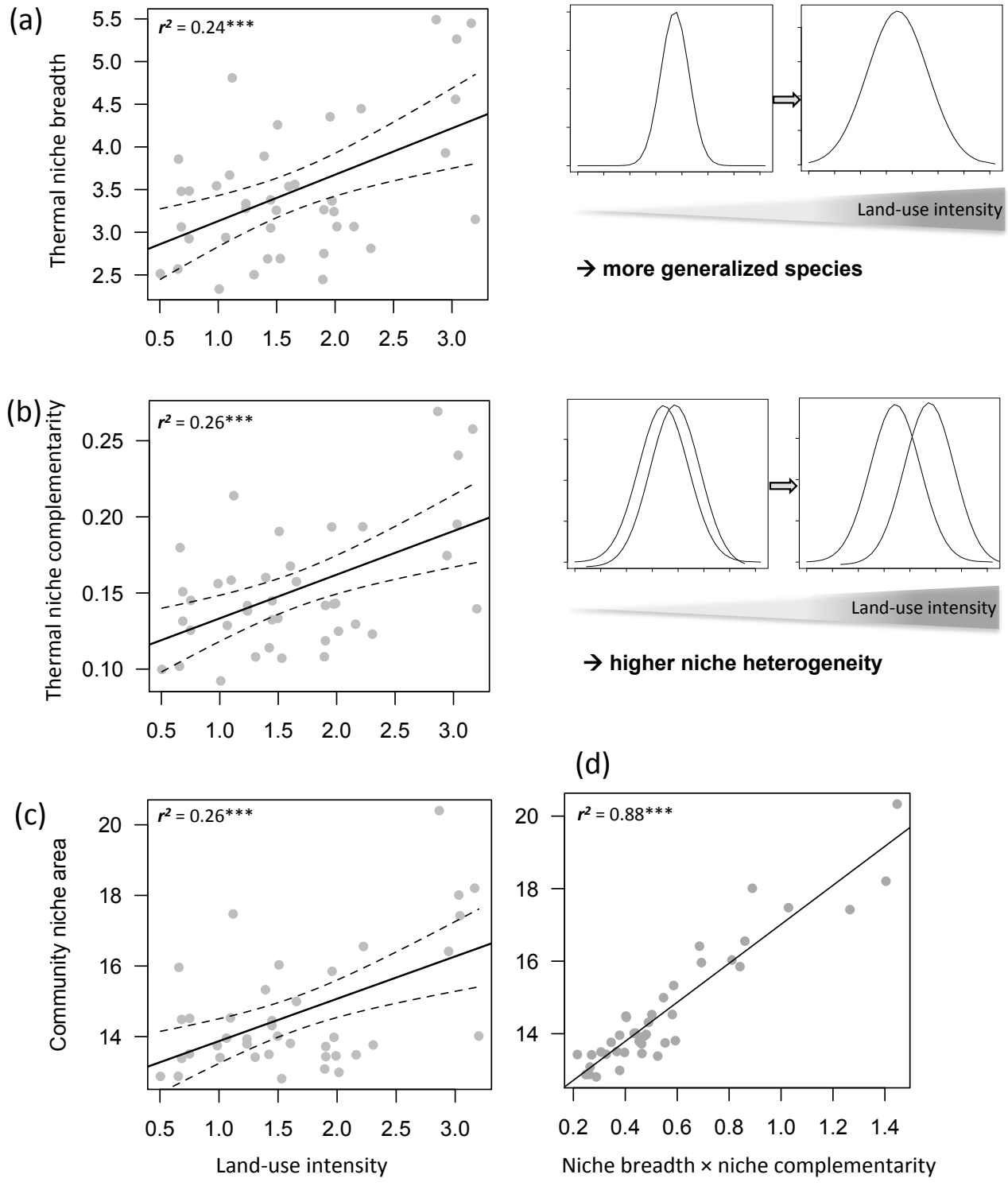

Fig. 2.3: Thermal niches of pollinators depending on land-use intensity. Effects of land-use intensity on thermal niche breadth (**a**), niche complementarity (**b**) and community niche area (**c**). Community niches were predicted by the product of niche breadth and complementarity (d). Dotted lines represent 95% confidence intervals.

Pollinator taxa differed in their thermal niches breadth and niche complementarity. Butterflies, hoverflies and bees had narrower niches and also a lower niche complementarity across the species than other flies, other hymenopterans and beetles (Table 2.2). Note that variation in niches is independent of number of species in each taxon, e.g. other hymenopterans contain relatively few species that were highly complementary whereas other flies or bees contained more species that were more similar in their thermal niches. Niche breadth und complementarity were even more variable at family level (see supplementary Fig. 2.2).

Table 2.2: Thermal niche of different pollinator taxa. Mean and standard deviation (sd) of niche breadth and niche complementarity for the six selected taxa.

	Niche breadth		Niche complementarity	
	mean	sd	mean	sd
butterflies	4.32	1.88	0.17	0.07
hoverflies	4.48	2.06	0.18	0.07
bees	4.50	1.85	0.18	0.07
other flies	4.53	2.42	0.21	0.14
other hymenopterans	4.56	1.73	0.23	0.12
beetles	5.11	2.27	0.22	0.11

Considering that flies became more dominant on intensively used plots we investigated whether effects of land-use intensity on the thermal responses of the community were mainly driven by flies or by other taxa. Niche breadth, complementarity and community niche area of flies alone were positively related to land-use intensity (Table 2.1), consistent with the trend found for all taxa. For the remaining taxa pooled, we found no effect of land-use intensity on thermal responses (see supplementary Table 2.3).

The species' body mass (log transformed) was a significant predictor of thermal optima: larger species preferred higher temperatures (lm: $F_{1,508} = 3.0$, $p = 0.002$, Fig. 2.4a). Flies (excluding hoverflies), hymenopterans (excluding bees) and beetles were comparably small, whereas bees and butterflies were almost two-fold larger (Fig. 2.4c). Pollinator communities in intensively used grasslands had a higher abundance of smaller species (lme, $F_{1,36} = 9.3$, $p = 0.004$, Fig. 2.4d) while heavier species (butterflies, hoverflies) became less common. There were no changes of body mass with land-use intensity within insect orders. Mean body mass differed between the two regions (lme, $F_{1,36} = 15.0$, $p < 0.001$), but the effect of land-use intensity was similar (interaction: $F_{1,36} = 3.3$, $p = 0.076$). Changes in thermal optima and with increasing land-use intensity were the same when we used measured body size instead of calculated body mass (see supplementary Fig. 2.3).

There was no correlation between the total abundances of species and their thermal optima (Spearman rank, $r_S = 0.04$, $p = 0.40$) and only a weak correlation between abundance and thermal niche breadth ($r_S = 0.16$, $p < 0.001$).

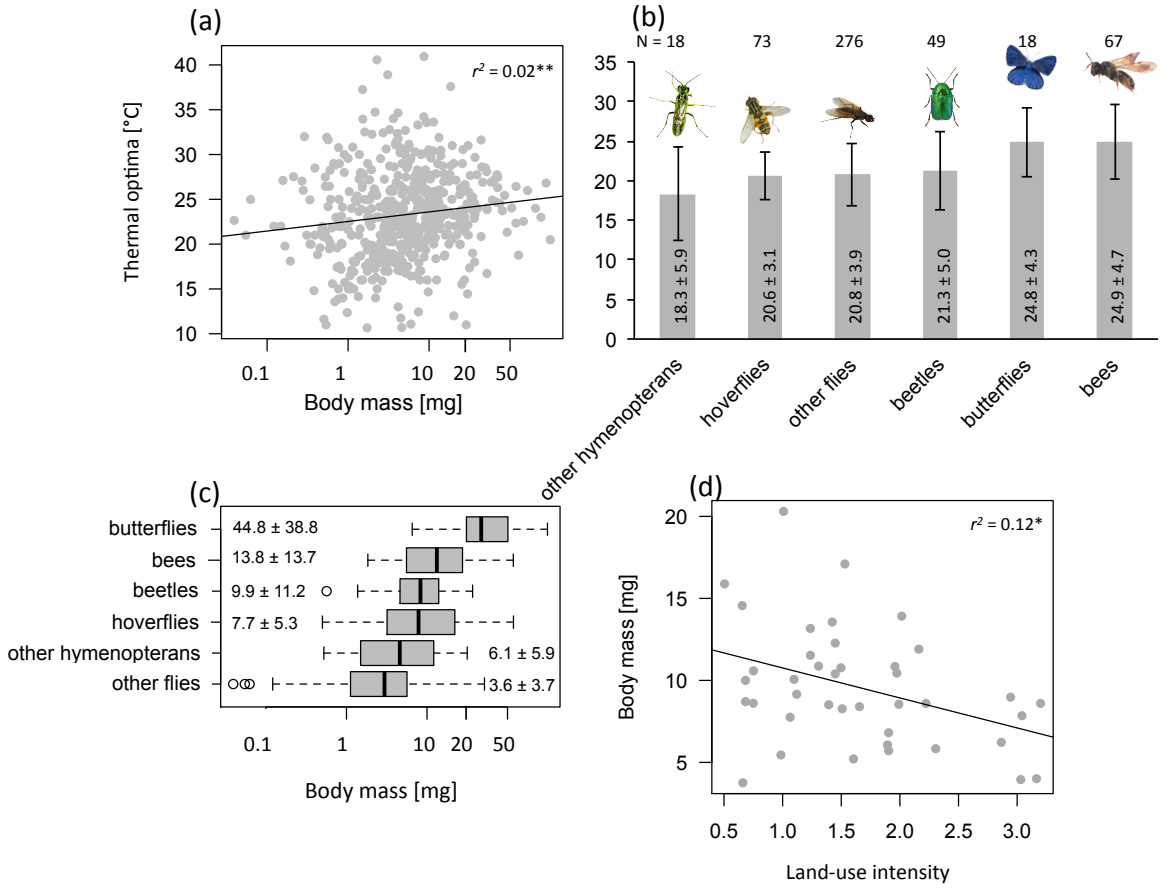

Fig. 2.4: Role of body mass of pollinators for thermal niches. Effects of body mass (**a**) and taxa (**b**) on thermal optima. Body mass of different taxa (mean ± sd) (**c**). Effects of land-use intensity on body mass (**d**).

Land-use effects were independent of the local microclimatic conditions. The mean summer air temperature was not correlated with land-use intensity (lm, $F_{1,32} = 0.4$, $p = 0.54$), and there was only a non-significant trend that pollinators in warmer grasslands had higher thermal optima ($F_{1,32} = 3.5$, $p = 0.070$). Hence, variation in thermal niches was independent of climatic conditions in the species habitats.

Discussion

In theory, response diversity – variation in species-specific reaction norms of functionally redundant species – is important in stabilizing ecosystem functioning over time and against environmental variability (Elmqvist *et al.* 2003, but see Cariveau *et al.* 2013). Based on thermal niches of a large number of pollinators, we modelled the thermal resilience of 40 local pollinator communities. We found that communities in intensively used grasslands had more variable temperature optima (hence, higher response diversity) as well as broader species-specific thermal niches (higher tolerance). Both contributed to a significant increase in projected thermal resilience with land-use intensity – an effect mainly driven by flies.

Ambient temperature appears as the main predictor of the general activity of insect pollinators – as expected for ectotherms – when observations cover a broad daytime temperature range from 5°C to 37°C (see also Huey & Pascual 2009). The effect exceeds by far the influence of variation in flower cover or flower diversity; both are known to affect pollinator abundance in other studies (Sjödin, Bengtsson & Ekbom 2007). In most pollinator studies temperature has not been taken into account, e.g. as a covariate in comparisons of visitation rates or pollinator diversities. Restricting the observation of pollinators to a narrowly defined range of intermediate temperatures, however, may limit undesired variation. The strong dependence of ectotherms such as insects to ambient temperatures makes them potentially vulnerable to climatic changes. Besides an increase in average temperature, climate change models highlight an increasing variation of summer and winter temperatures (Schär *et al.* 2004) and a higher frequency of extreme weather events. Given our findings, we might expect pollinator species at intermediate latitudes to be less susceptible to stress due to temperature variation, while tropical and desert species may not tolerate further warming it such warming exceed critical temperature thresholds (Deutsch *et al.* 2008; Kingsolver *et al.* 2013). Generally, broader thermal niches and not only higher thermal optima may buffer species against climate change impacts (Buckley & Kingsolver 2012; Huey *et al.* 2012). Therefore, community resilience benefits from species with broad niches, but also from species covering very different niches (high complementarity). Although species with lower thermal optima became more common in grasslands with land-use intensity, projected activities of pollinator communities during particularly warm temperatures (35°C to 40°C) were still not reduced compared to low-intensity grasslands. The increase in thermal niche breadth thus compensated for a directional shift towards colder thermal optima. Therefore, in these highly diverse communities in meadows and pastures, climate warming does not appear to restrict the activity of pollinators and their potential services.

Interestingly, variation in thermal niches in this study did not correspond to climatic differences across the grasslands (which were mostly relatively similar) nor between the regions. Instead, the composition of pollinator species was driven by other environmental filters related to land-use intensity, and resulting effects on thermal community niches appeared as by-product of other traits such as body mass and taxonomic constraints.

Changes in composition of functional traits have been reported from different communities and contexts, usually defined for morphological or life-history traits (e.g. Williams *et al.* 2010). High land-use intensity has been found to reduce the diversity of species and their functional traits in several studies, a trend that has been termed 'functional homogenization' (Flynn *et al.* 2009; Laliberté *et al.* 2010). Characteristic traits of communities may thus act as land-use indicators (Pfestorf *et al.* 2013). With increasing land-use intensity, habitat or food specialists are often found to decline whereas generalists increase (Clavel, Romain & Devictor 2010). In parallel to the increase in generalized species (in terms of their thermal niche) in the grasslands investigated here, generalized butterflies (in terms of larval host plants) and other pollinators (in terms of flowers visited) became more common with increasing land-use intensity, while specialists declined (Börschig *et al.* 2013; Weiner *et al.* 2014). In arable land, insecticides additionally affect life-history traits of bees with negative consequences for pollination (Brittain & Potts 2011).

Variation in thermal optima of pollinators was significantly related to body mass: lighter insects preferred lower temperatures. The increased surface / volume ratio of small and light animals could trigger higher water loss rates (Hadley 1994), which corresponds to an avoidance of warmer temperatures. This rule may only apply to sufficiently warm conditions, as cold temperatures in higher altitudes or latitudes may represent an energetic threshold restricting smaller bodied pollinators. Moreover, most flies in our study are relatively small compared to bees and butterflies. Colder community thermal optima in intensively used grasslands thus correspond to the dominance of small flies and the loss of pollinators that prefer warmer temperatures. The shift of body size with land-use intensity towards a prevalence of small-sized species is a pattern that is already known from beetle assemblages (Braun, Jones & Perner 2004; Magura, Tóthmérész & Lövei 2006).

The abundance of butterflies and bees (in one region) decreased with the land-use intensity, whereas flies compensated these losses by an increasing abundance. Thus total diversity remained unaffected. Differences in community niches thus did not reflect pure diversity effects rather than changes in species composition. The contrasting responses of declining plant and stable pollinator diversity

confirmed earlier findings from the plots and other grasslands in the Biodiversity Exploratories (Weiner *et al.* 2014).

A special role comes to flies in our study as they are representing the largest share of the pollinator community particularly in intensively used grasslands. Especially in high altitude or latitude systems, or for example in island systems where other pollinators are rare, flies are known to be crucial for pollination (Ssymank *et al.* 2008). But their role for other ecosystems is poorly investigated and thus probably largely underestimated. Flies are generally important pollinators, characterized by a high taxonomic diversity (Larson, Kevan & Inouye 2001) and high interaction frequencies (Vazquez, Morris & Jordano 2005) and make a major contribution to plant diversity as well as agricultural production. Not only occasionally studied syrphid-flies, but also often more numerous non-syrphid flies contribute to pollination (Orford, Vaughan & Memmott 2015). Flies are known to respond differently to environmental disturbance than for example bees (Kearns 2001; Biesmeijer *et al.* 2006). Our results suggest that they also differ in thermal niches from other pollinators and, therefore, could maintain pollination services at low temperatures where bees or butterflies are inactive. Their dominance compared to other pollinators, but also shifts of species composition within the flies contributed to the higher thermal resilience of the communities. The same effects of land-use intensity on thermal niches that we found for all pollinators together also apply to the flies alone, but not to the remaining taxa (although these were similarly variable in niche breadth and niche complementarity). This shows that the impact of land use on thermal niches is an effect within the heterogeneous flies rather than an effect of having more flies. Therefore flies could be highlighted as a main stabilizing factor of pollinator communities in managed grassland ecosystems. Flies are more generalized pollinators than most other taxa (Weiner *et al.* 2014) and may thus be less affected by declining plant species diversity. The diversity of hoverflies has declined much less (and partly even increased) in some regions over the last decades compared to the more specialized bees (Biesmeijer *et al.* 2006). Open flowers such as Asteraceae became much more common in intensively used grasslands although total plant diversity strongly decreased with land-use intensity in our study. They provide nectar and pollen that is easily accessible for short-tongued flies. In addition to adult diets, flies could additionally profit from increases in various larval resources and habitats (Jauker *et al.* 2009).

Differences in thermal niches between pollinator taxa are relevant for maintaining pollination across a range of weather conditions, which has been described in several case studies. For example, Vicens and Bosch (2000) showed that *Osmia cornuta* had greater weather tolerance than other species pollinating apple flowers. In almond orchards honeybees were replaced from wild pollinators at high wind speeds (Brittain, Kremen & Klein 2013). Weather also plays an important role in the pollination and yield of

high bush blueberry, corresponding to variation in pollinator community composition (Tuell and Isaakcs 2010). Fründ *et al.* (2013) used between one and five pollinator species in experimental cages to study their pollination of several plants, and also fitted temperature activity curves for each pollinator species. The temperature range for which at least one species was active and could provide pollination services increased with the diversity of the community, which could contribute to a higher pollination success.

Other studies showed strong negative effects on pollinators or their pollination services as a response to habitat conversion or fragmentation (Steffan-Dewenter & Westphal 2007; Potts *et al.* 2010). The gradient in our study represents quantitative differences in grassland management intensity (fertilization, mowing, grazing), but did not involve unmanaged fallows or habitat conversion to arable land that are usually associated with strong biodiversity losses. Therefore, we caution against generalizations of our findings about resilient communities: in scenarios of stronger biodiversity losses and for taxa that suffer more substantially from land use, losses in stability are expected.

We conclude that an increase in thermal resilience of pollinators in intensively used grasslands is mainly driven by flies that profit from land use and compensate for losses of other taxa. The higher thermal resilience suggests that across the entire temperature range in the vegetation period, at least some species may continue to perform their functions. This highlights the relevance of response diversity for the resilience of ecosystems against variable conditions. The diversity of pollinators was high in all grasslands investigated here. However, in ecosystems with high losses of species and more severe human impacts, a more limited response diversity could put the maintenance of ecosystem functions at risk.

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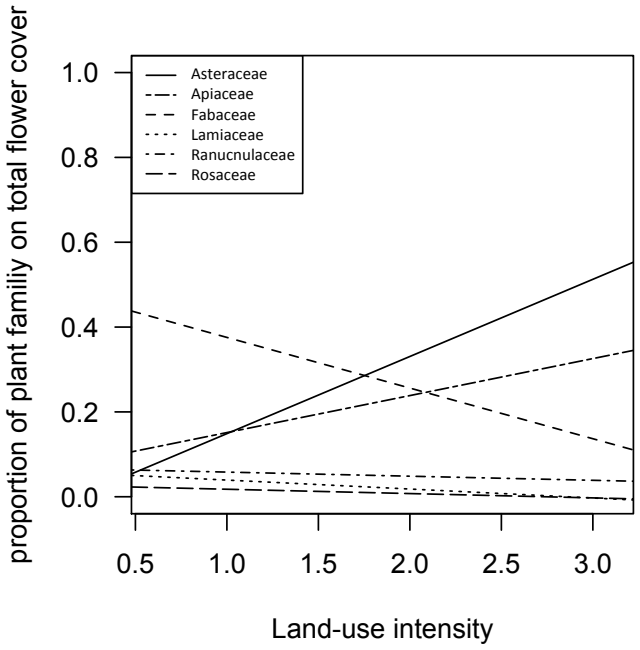
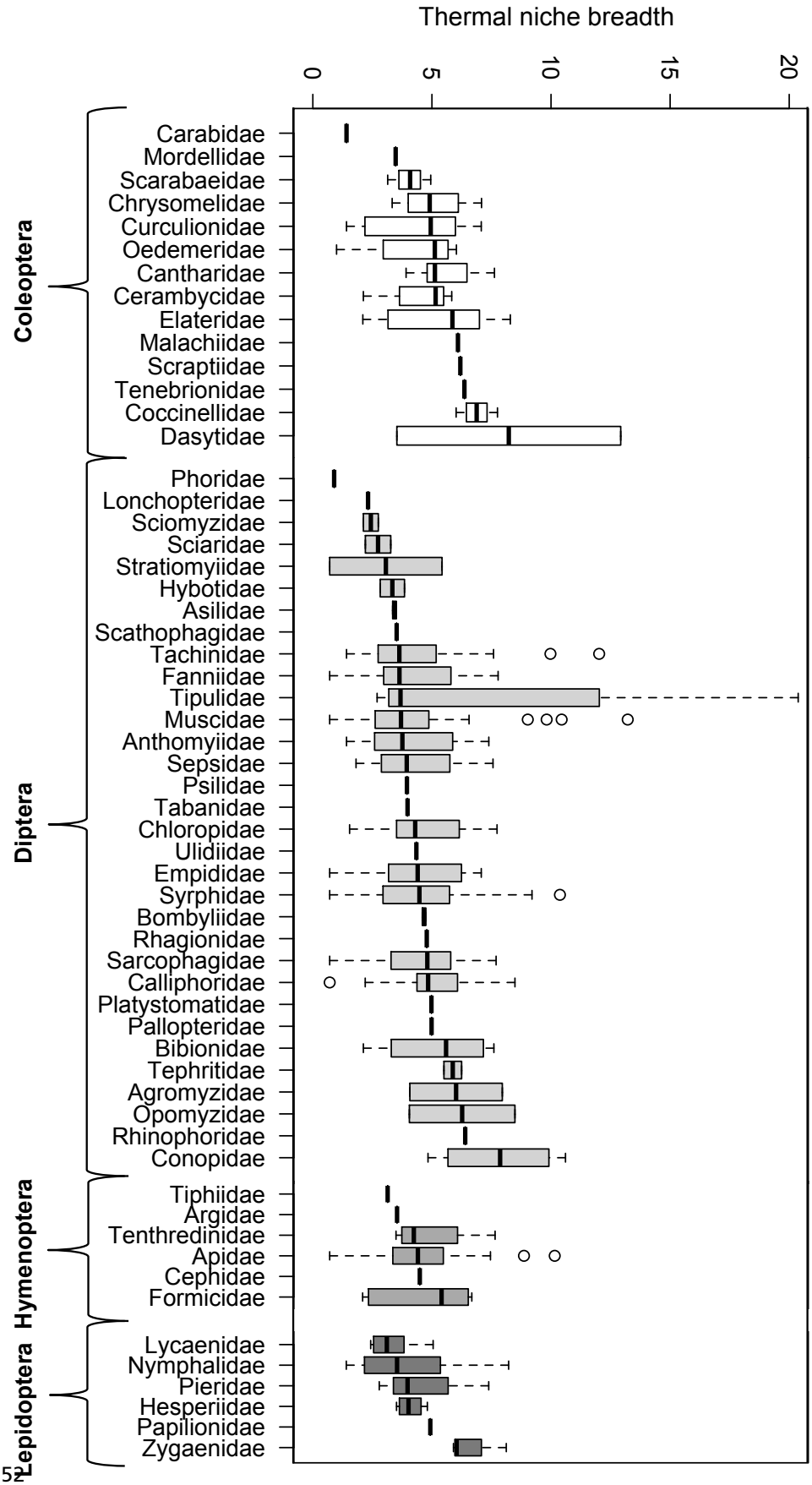
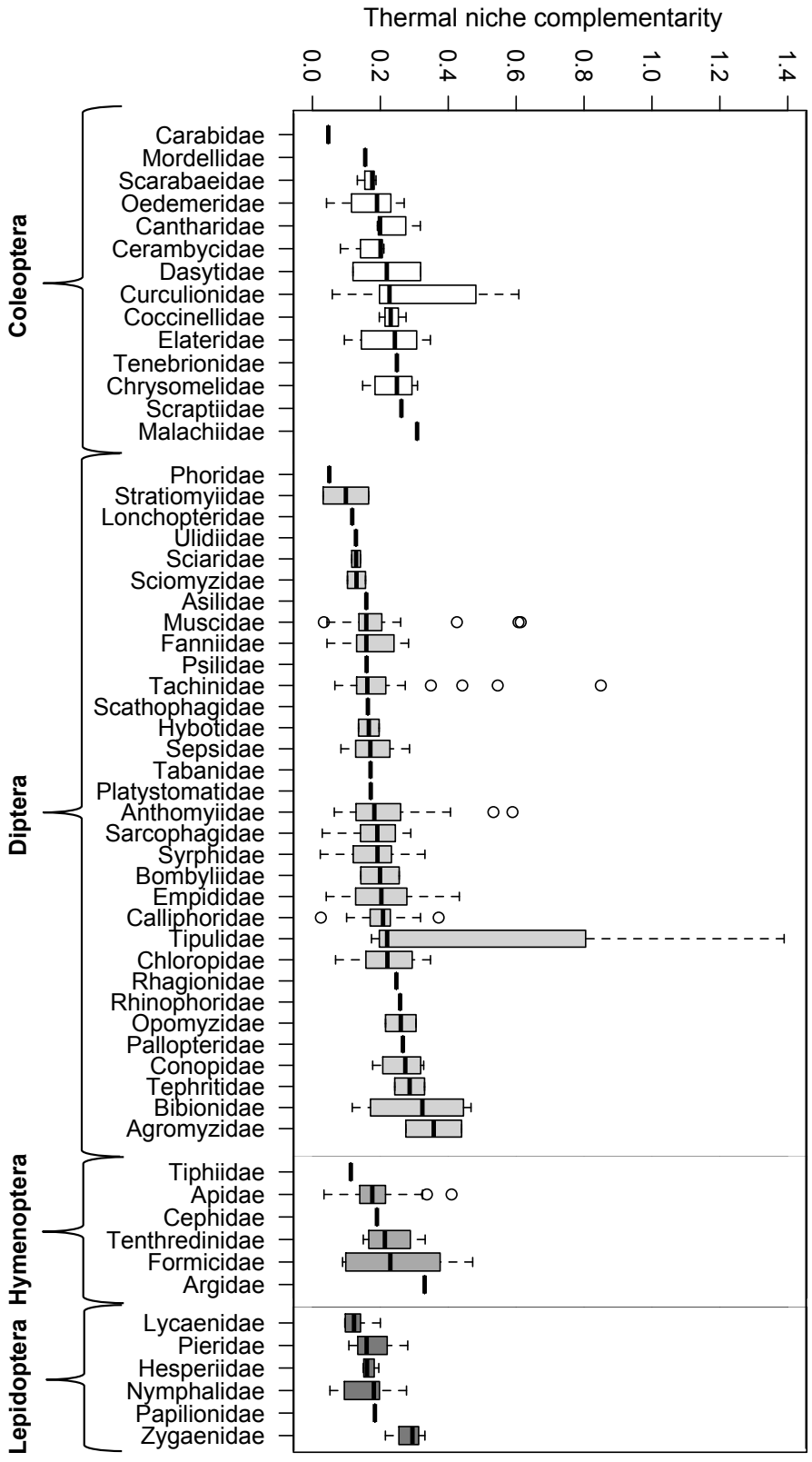
Supplementary Information

Supplementary Fig. 2.1: Changes in plant community with land-use intensity. The proportions of plant families of the total flower cover per plot and their changes with land-use intensity.

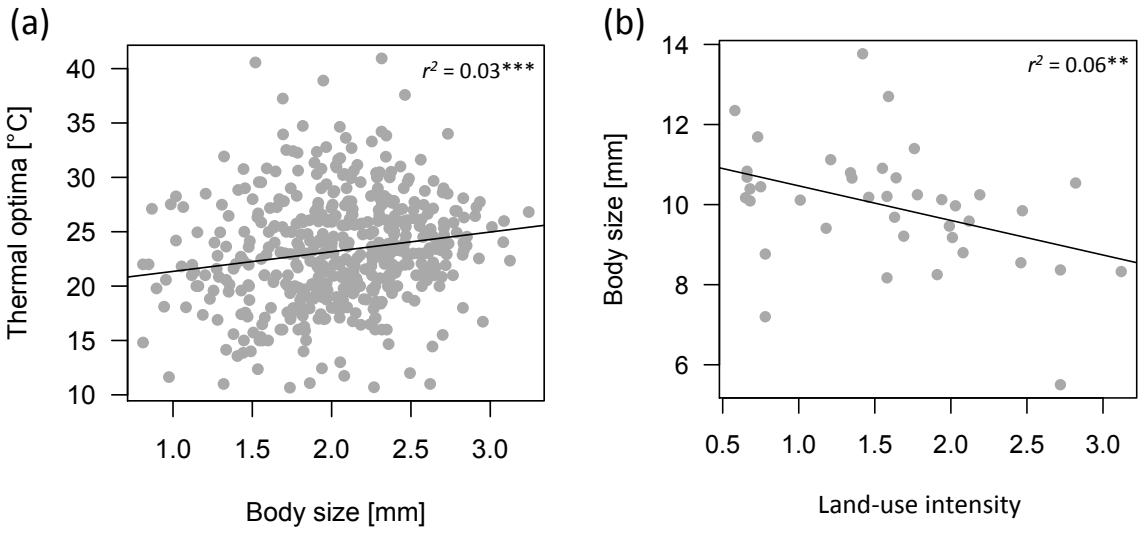


Supplementary Fig. 2.2: Thermal niches of pollinator families. Variation in niche breadth and thermal niche complementarity of pollinator families within each of the four insect orders. Boxplots represent median, upper and lower quartiles and standard deviation of the species' values in each family.

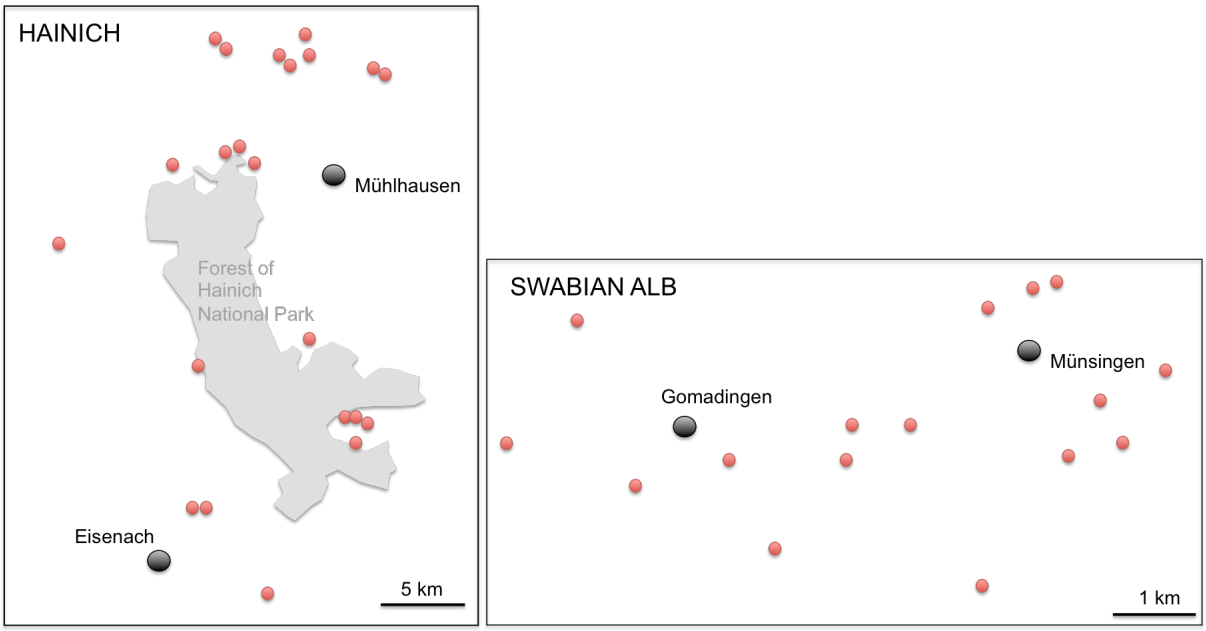



Thermal niche complementarity
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Coleoptera
Carabidae
Mordellidae
Scarabaeidae
Oedemeridae
Cantharidae
Cerambycidae
Dasytidae
Curculionidae
Coccinellidae
Elateridae
Tenebrionidae
Chrysomelidae
Scraptiidae
Malachiidae
Diptera
Phoridae
Stratiomyiidae
Lonchopteridae
Ulidiidae
Sciaridae
Sciomyzidae
Asilidae
Muscidae
Fanniidae
Psilidae
Tachinidae
Scathophagidae
Hybotidae
Sepsidae
Tabanidae
Platystomatidae
Anthomyiidae
Sarcophagidae
Syrphidae
Bombyliidae
Empididae
Calliphoridae
Tipulidae
Chloropidae
Rhagionidae
Rhinophoridae
Opomyzidae
Pallopteridae
Conopidae
Tephritidae
Bibionidae
Agromyzidae
Hymenoptera
Tiphiidae
Apidae
Cephidae
Tenthredinidae
Formicidae
Argidae
Lepidoptera
Lycaenidae
Pieridae
Hesperiidae
Nymphalidae
Papilionidae
Zygaenidae

Supplementary Fig. 2.3: Relationship between body size of pollinators, thermal niches and land-use intensity. (**a**) Effect of body size on thermal optima of species and (**b**) effects of land-use intensity on weighted mean body size across plots.



Supplementary Fig. 2.4: Map of grassland plots. Location of the grassland plots used for pollinator sampling in Hainich (Thüringen, central Germany) and Swabian Alb (Baden-Württemberg, southwest Germany).



Supplementary Table 2.1: Compositional changes of plant communities with land-use intensity. Changes in the proportion of plant families of total flower cover per plot with increasing land-use intensity, assessed with linear models for each family. *N* = number of plots in which the family occurred. Predictors with $p < 0.05$ were considered to be significant (boldface).

	F	*p*	*N*
Asteraceae	13.3	**< 0.001**	35
Apiaceae	2.5	0.122	35
Fabaceae	4.3	**0.046**	37
Lamiaceae	3.9	0.055	34
Ranunculaceae	0.2	0.680	26
Rosaceae	2.2	0.151	23

Supplementary Table 2.2: Determinants of thermal niches of pollinator communities in 2008. Effects of land-use intensity (LUI), region and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Land use intensity was averaged over the years 2007 and 2008. Results are from linear mixed models. Predictors with $p < 0.05$ were considered to be significant (boldface).

	df_{num}	df_{den}	*F*	*p*
Thermal optima				
LUI	1	65	< 0.1	0.881
Region	1	65	< 0.1	0.849
LUI × Region	1	65	0.5	0.498
Diversity	1	65	< 0.1	0.922
Thermal niche breadth				
LUI	1	65	3.3	0.075
Region	1	65	1.4	0.248
LUI × Region	1	65	0.4	0.524
Diversity	1	65	7.1	**0.010**
Thermal niche complementarity				
LUI	1	65	3.1	0.083
Region	1	65	1.2	0.279
LUI × Region	1	65	0.6	0.452
Diversity	1	65	6.1	**0.016**
Community niche area				
LUI	1	65	1.8	0.185
Region	1	65	< 0.1	0.826
LUI × Region	1	65	1.2	0.279
Diversity	1	65	24.9	**< 0.001**

In 2008, 588 pollinator species were observed on 70 grassland plots in Hainich and Swabian Alb with the same method of pollinator sampling method like in this study. 47 of the 70 plots were just observed one day (6h) at different times of the year and different times of the day.

Supplementary Table 2.3: Determinants of thermal niches of pollinator communities without flies. Effects of land-use intensity (LUI), region and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Results are from linear mixed models. Flies were excluded from the analysis. Predictors with $p < 0.05$ were considered to be significant and bold print.

	df_{num}	df_{den}	F	p
Thermal optima				
LUI	1	35	7.9	**0.008**
Region	1	35	0.3	0.598
LUI × Region	1	35	0.3	0.580
Diversity	1	35	0.6	0.446
Thermal niche breadth				
LUI	1	34	2.2	0.144
Region	1	34	1.1	0.299
LUI × Region	1	34	0.2	0.658
Diversity	1	34	0.1	0.806
Thermal niche complementarity				
LUI	1	34	2.7	0.11
Region	1	34	1.1	0.313
LUI × Region	1	34	0.2	0.653
Diversity	1	34	0.1	0.790
Community niche area				
LUI	1	34	1.7	0.200
Region	1	34	0.5	0.501
LUI × Region	1	34	< 0.1	0.961
Diversity	1	34	< 0.1	0.731

Supplementary Table 2.4: Long term effects on thermal niches of pollinator communities. Effects of land-use intensity (LUI), region and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Land use intensity was averaged over the years 2006 to 2012. Results are from linear mixed models. Predictors with $p < 0.05$ were considered to be significant (boldface).

	df_{num}	df_{den}	*F*	*p*
Thermal optima				
LUI	1	35	12.1	**0.001**
Region	1	35	1.1	**0.002**
LUI × Region	1	35	10.3	**0.003**
Diversity	1	35	0.1	0.804
Thermal niche breadth				
LUI	1	35	12.7	**0.001**
Region	1	35	0.1	0.798
LUI × Region	1	35	1.2	0.282
Diversity	1	35	0.2	0.689
Thermal niche complementarity				
LUI	1	35	13.9	**< 0.001**
Region	1	35	0.3	0.567
LUI × Region	1	35	2.2	0.145
Diversity	1	35	0.2	0.67
Community niche area				
LUI	1	35	15.5	**< 0.001**
Region	1	35	< 0.1	0.853
LUI × Region	1	35	1.2	0.287
Diversity	1	35	0.4	0.530

Supplementary Table 2.5: Plots sampled. Number of surveys (6 h) per plot in Alb and Hainich.

Plot (Alb)	Number of surveys	Plot (Hainich)	Number of surveys
AEG01	4	HEG01	6
AEG02	4	HEG02	4
AEG03	10	HEG03	2
AEG04	4	HEG04	1
AEG05	10	HEG05	5
AEG06	3	HEG06	5
AEG07	11	HEG07	10
AEG08	7	HEG08	9
AEG09	13	HEG09	2
AEG16	4	HEG10	2
AEG17	6	HEG15	1
AEG22	1	HEG17	2
AEG26	6	HEG18	2
AEG27	2	HEG20	3
AEG29	4	HEG24	2
AEG48	8	HEG26	2
		HEG28	2
		HEG29	2
		HEG31	4
		HEG41	3
		HEG42	2
		HEG44	1
		HEG47	2
		HEG48	4

Supplementary Table 2.6: Determinants of thermal niches of a subset of pollinator communities. All pollinator species with less than 5 individuals were excluded from the analysis to avoid inaccurate thermal niches resulting from only few data points. Effects of land-use intensity (LUI), region and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Results are from linear mixed models. Flies were excluded from the analysis. Predictors with $p < 0.05$ were considered to be significant (boldface).

	df_{num}	df_{den}	F	p
Thermal optima				
LUI	1	36	11.3	**0.002**
Region	1	36	8.61	**0.006**
LUI × Region	1	36	16.71	**< 0.001**
Thermal niche breadth				
LUI	1	36	9.2	**0.005**
Region	1	36	0.1	0.761
LUI × Region	1	36	1.7	0.203
Thermal niche complementarity				
LUI	1	36	10.6	**0.002**
Region	1	36	< 0.1	0.96
LUI × Region	1	36	3.5	0.071
Community niche area				
LUI	1	36	4.1	**0.049**
Region	1	36	< 0.1	0.924
LUI × Region	1	36	< 0.1	0.851

Supplementary Table 2.7: Determinants of unweighted thermal niches of pollinator communities. Effects of land-use intensity (LUI), region and pollinator diversity ($e^{H'}$) on thermal responses of pollinators. Results are from linear mixed models. Thermal responses are not weighted by abundance of the individuals considering that frequency can change. Predictors with $p < 0.05$ were considered to be significant (boldface).

	dfnum	dfden	*F*	*p*
Thermal optima (unweighted)				
Taxa	5	168	14.8	**< 0.001**
LUI	1	36	8.7	**0.006**
Region	1	36	2.6	0.114
LUI × Region	1	36	< 0.1	0.953
Diversity	1	168	1.9	0.170
Thermal niche breadth (unweigthed)				
Taxa	5	141	16.6	**< 0.001**
LUI	1	36	10.3	**0.003**
Region	1	36	3.0	0.093
LUI × Region	1	36	0.1	0.740
Diversity	1	141	< 0.1	0.913
Thermal niche complementarity (unweighted)				
Taxa	5	141	19.8	**< 0.001**
LUI	1	36	16.8	**< 0.001**
Region	1	36	1.4	0.245
LUI × Region	1	36	0.1	0.768
Diversity	1	141	0.1	0.733

Supplementary Data 2.1: Flower resources on the plots. Mean number of flowering plant species, mean flowering area on the plots, mean effective plant species diversity ($e^{H'}$, calculated with the number of plant species per plot and their proportion of flowering area on total flowering area per plot) and number of surveys for all plots in both regions. At each survey number and size of all florescence of all flowering plant species were recorded.

region	plot_ID	plant species richness	flowering area [cm^2]	$e^{H'}$	Number of surveys
Schwäbische	AEG01	14	128416.63	2.85	4
Alb	AEG02	13.25	47720.7	2.77	4
	AEG03	17.5	15151.41	5.29	8
	AEG04	12.75	8517.1	3.5	4
	AEG05	12.5	57591.9	2.72	8
	AEG06	12	47264.16	3.63	5
	AEG07	19.38	12808.54	6.54	8
	AEG08	22.17	12222.24	5.93	6
	AEG09	20.5	23384.13	5.91	8
	AEG16	15	45058.81	3.69	2
	AEG17	15.25	50113.6	2.75	4
	AEG22	21	20945.63	9.01	1
	AEG26	22.67	15780.62	5.93	3
	AEG27	29	13366.84	7.36	1
	AEG29	18.5	7418.28	4.6	2
	AEG48	22.67	11041.3	8.15	3
	AEG48	25.5	17671.34	6.42	2
Hainich	HEG01	9.75	5963.78	3.6	4
	HEG02	6.4	20075.12	1.58	5
	HEG03	8	6117.21	3.17	3
	HEG04	3.5	908.24	2.28	2
	HEG05	9.67	15052.15	1.48	3
	HEG06	12	13036.5	2.91	6
	HEG07	13.43	37595.72	2.65	7
	HEG08	13.83	33123.08	4.53	6
	HEG09	11.5	595.92	5.59	2
	HEG10	15	13170.25	5.97	1
	HEG15	8	84022.79	2.32	1
	HEG17	18	13450.91	6.33	1
	HEG18	34	92080.54	2.79	1
	HEG20	26	99764.82	5.07	2
	HEG26	10	3979.15	3.65	1
	HEG28	20	43606.59	5.15	1
	HEG29	16	13227.07	5.47	1
	HEG31	16	12029.4	3.58	2
	HEG41	17.5	15341.1	6.35	2
	HEG42	24	73147.96	5.39	1
	HEG44	29	104939	3.94	1
	HEG47	13	41400.84	2.37	1
	HEG48	16.67	31327.44	3.94	3

Supplementary Data 2.2: List of pollinator species. For every species the associated insect order is given as well as measured body size and calculated body mass (from conversion equations). Some species are assigned to a species aggregate (agg.). Missing values (two cases) are marked as "NA".

order	species	body size [mm]	predicted body mass [mg]
Coleoptera	*Agriotes gallicus*	7.44	6.72
	Agriotes obscurus	11.92	21.64
	Agriotes sputator	6.24	4.36
	Amara familiaris	6.73	5.25
	Anaspis frontalis	3.8	1.31
	Athous bicolor	9.05	10.9
	Athous haemorrhoidalis	12.96	26.69
	Bruchus rufimanus	5.16	2.74
	Cantharis flavilabris	5.92	3.83
	Cantharis fusca	15.01	38.59
	Cantharis livida	12.45	24.13
	Cetonia aurata	18.26	63.29
	Ceutorhynchus assimilis	2.65	0.55
	Chrysanthia geniculata	6.46	4.75
	Cidnopus aeruginosus	10.55	15.96
	Coccinella septumpunctata	8.08	8.24
	Cryptocephalus hypochoeridis	5.57	3.3
	Cryptocephalus populi	3.53	1.09
	Cryptocephalus sericeus	6.57	4.95
	Cryptocephalus vittatus	4.9	2.42
	Dasytes niger	4.74	2.23
	Dasytes plumbeus	4.57	2.04
	Dinoptera collaris	8.94	10.58
	Eusomus ovulum	7.1	5.99
	Glocianus punctiger	2.87	0.67
	Harpalus affinis	9.89	13.59
	Hemicrepidius hirtus	10.55	15.96
	Hemicrepidius niger	10.9	17.31
	Hoplia argentea	10.63	16.26
	Hypoganus inunctus [1)]	11	7.71
	Lagria hirta	7.9	7.79
	Malachius bipustulatus	6.94	5.66
	Mordellistena brevicauda	4.23	1.69
	Mordellochroa abdominalis	5.86	3.74
	Oedemera femorata	9.52	12.36
	Oedemera lurida	6.42	4.68
	Oedemera virescens	7.63	7.15
	Phyllobius betulinus	6.95	5.68
	Phyllobius pyri	6.44	4.71
	Phyllopertha horticola	10.22	14.75
	Phyllotreta vittula	3.6	1.15
	Poecilus versicolor	11.4	19.36
	Propylea quatuordecimpunctata	10.84	17.08
	Pseudovadonia livida	8	8.04
	Rhagonycha fulva	4.97	2.5
	Rhagonycha limbata	7.38	6.59
	Stenurella melanura	8.42	9.12
	Tytthaspis sedecimpunctata	3.66	1.19
	Zacladus geranii	2.77	0.61
Diptera	*Adia cinerella*	5.31	1.36
	Allophorocera ferruginea	8.17	3.86
	Ancistrocerus nigricornis	13.5	13.1
	Angioneura fimbriata	4.39	0.86
	Anthomyia liturata	5.69	1.61
	Azelia trigonica	3.06	0.36
	Bellardia bayeri	7.39	3.03
	Bellardia pandia	10.43	6.99
	Bellardia viarum	8.38	4.11
	Bellardia vulgaris	8.58	4.35
	Bibio johannis	6.62	2.32

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Bibio leucopterus*	9.53	5.61
	Bicellaria spuria	3.51	0.5
	Bicellaria sulcata	3.63	0.54
	Billaea triangulifera	9.83	6.05
	Bithia spreta	8.44	4.18
	Blaesoxipha laticornis	6.74	2.42
	Blaesoxipha plumicornis	6.11	1.91
	Blondelia nigripes	8.5	4.25
	Bombylius atra	5.96	1.8
	Bombylius major	9.96	6.25
	Bombylius venosus	10.14	6.53
	Botanophila biciliaris	7.04	2.69
	Botanophila brunneilinea	6.9	2.56
	Botanophila discreta	6.81	2.48
	Botanophila fugax	5.77	1.66
	Botanophila seneciella	4.78	1.05
	Botanophila striolata	6.06	1.87
	Botanophila varicolor	8.14	3.83
	Brachicoma devia	8.45	4.19
	Calliopum aeneum [2)]	4.25	0.79
	Calliphora vicina	10.33	6.83
	Calliphora vomitoria	11.9	9.63
	Calythea nigricans	4.91	1.12
	Cheilosia albitarsis	9.91	6.17
	Cheilosia barbata	9.4	5.43
	Cheilosia canicularis	13.69	13.55
	Cheilosia gigantea	9.73	5.9
	Cheilosia impressa	6.77	2.45
	Cheilosia lenis	10.32	6.81
	Cheilosia pagana	8.02	3.69
	Cheilosia ranunculi	10.6	7.27
	Cheilosia scutellata	9.98	6.28
	Cheilosia soror	10.3	6.78
	Cheilosia vernalis	7.48	3.12
	Cheilosia vulpina	10.51	7.12
	Chloromyia formosa	7.14	2.78
	Chlorops pumilionis	3.88	0.64
	Chrysogaster basalis	8.12	3.8
	Chrysogaster solstitialis	8.49	4.24
	Chrysotoxum arcuatum	13.45	12.98
	Chrysotoxum bicinctum	12.03	9.89
	Chrysotoxum festivum	14.01	14.34
	Chrysotoxum vernale	13.05	12.06
	Chyliza extenuata	6.79	2.47
	Coenosia infantula	3.86	0.63
	Coenosia pedella	3.16	0.39
	Coenosia pumila	4.27	0.8
	Coenosia tigrina	5.86	1.73
	Coptocephala rubicunda	5.25	1.32
	Cynomya mortuorum	11.69	9.22
	Dasysyrphus albostriatus	10.46	7.04
	Dasysyrphus hilaris	11.19	8.29
	Dasysyrphus pinastri	9.61	5.73
	Dasysyrphus tricinctus	11.64	9.13
	Delia coarctata	6.43	2.16
	Delia floricola	6.4	2.14
	Delia florilega	4.23	0.78
	Delia lophota	6.45	2.18
	Delia platura	4.51	0.92
	Delia radicum	6.72	2.4
	Dexia rustica	11.8	9.44
	Dilophus febrilis	8.28	3.99
	Dilophus femoratus	6.22	1.99
	Dilpohus humeralis	4.92	1.13
	Dinera ferina	11.93	9.69

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Dioctria atricapilla*	2.95	0.33
	Diplonerva nitidula	7.22	2.86
	Dolichpus ungulatus	8.24	3.94
	Drino vicina	5.28	1.34
	Ecoptomera pallescens	6.92	2.58
	Eggisops pecchiolii	4.04	0.7
	Eloceria delecta	10.96	7.88
	Empis aestiva	4.24	0.79
	Empis caudatula	4.73	1.03
	Empis ciliata	9.62	5.74
	Empis dimidiata	6.81	2.48
	Empis femorata	7.77	3.42
	Empis genualis	3	0.34
	Empis grisea	7.38	3.02
	Empis laminata	4.18	0.76
	Empis lepidopus	5.55	1.51
	Empis livida	10.42	6.97
	Empis mariae [3)]	2.7	4.88
	Empis nigripes	3.7	0.57
	Empis nuntia	4.08	0.72
	Empis opaca	8.45	4.19
	Empis pseudonuntia	5.84	1.71
	Empis sericans [4)]	9	4.88
	Empis stercorea	5.7	1.61
	Epicampocera succinata	8.35	4.07
	Episyrphus balteatus	11.06	8.06
	Eriothrix rufomaculatus	9.32	5.32
	Eristalinus sepulchralis	10.86	7.71
	Eristalis abusiva	11.72	9.28
	Eristalis arbustorum	11.04	8.03
	Eristalis horticola	12.59	11.05
	Eristalis nemorum	13.47	13.03
	Eristalis similis	14.12	14.61
	Eristalis tenax	14.98	16.88
	Estheria cristata	10.95	7.87
	Eudasyphora cyanicolor	7.96	3.62
	Eumerus strigatus	8.08	3.76
	Eupeodes bucculatus	10.13	6.51
	Eupeodes corollae	10.29	6.76
	Eupeodes latifasciatus	9.28	5.26
	Eupeodes luniger	9.88	6.13
	Eurithia connivens	9.94	6.22
	Eurychaeta palpalis	11.52	8.9
	Exorista mimula	6.94	2.6
	Exorista rustica	8.87	4.71
	Exorista tubulosa	9.2	5.15
	Fannia armata	4.67	1
	Fannia canicularis	6.27	2.03
	Fannia latipalpis	5.54	1.51
	Fannia postica	4.95	1.15
	Fannia rondanii	3.7	0.57
	Fannia serena	4.41	0.87
	Fannia sociella	4.77	1.05
	Fannia umbrosa	4.37	0.85
	Geomyza tripunctata	3.43	0.47
	Gonia capitata	13.3	12.63
	Gonia geniculata	12.38	10.61
	Graphomya maculata	8.36	4.08
	Haematopota pluvialis	11.46	8.79
	Hebecnema nigra	4.84	1.09
	Hebecnema umbratica	4.84	1.09
	Hebecnema vespertina	4.69	1.01
	Helina impuncta	7.77	3.42
	Helina latitarsis	6.16	1.95
	Helina laxifrons	7.47	3.11

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Helina obscurata*	6.8	2.47
	Helina quadrum	7.81	3.46
	Helina reversio	5.71	1.62
	Helina trivittata	7.59	3.23
	Helophilus hybridus	15.44	18.17
	Helophilus pendulus	13.22	12.45
	Helophilus trivittatus	15.93	19.61
	Heringia latitarsis	7.2	2.84
	Heterostylodes nominabilis	3.61	0.53
	Heterostylodes obscurus	4.18	0.76
	Hilara longivittata	3.34	0.44
	Hybomitra ciureai	14.58	15.8
	Hydrophoria silvicola	5.91	1.76
	Hydrotaea albipuncta	4.74	1.03
	Hydrotaea dentipes	8.23	3.93
	Hydrotaea meteorica	5.42	1.43
	Hydrotaea pandellei	5.99	1.82
	Hydrotaea parva	3.75	0.59
	Hylemya urbica	5.68	1.6
	Hylemya vagans	8.6	4.37
	Hylemya variata	6.13	1.92
	Hylemyza partita	4.14	0.74
	Leptogaster cylindrica	10.85	7.69
	Limnia unguicornis	5.59	1.54
	Linnaemya picta	11.79	9.42
	Loewia phaeoptera	4.28	0.81
	Lonchoptera bifurcata	2.45	0.21
	Lophosceles cinereiventris	5.61	1.55
	Lucilia caesar	8.69	4.49
	Lucilia illustris	9.49	5.56
	Lucilia sericata	8.01	3.68
	Lucilia silvarum	9.47	5.53
	Lydella stabulans	7.12	2.77
	Machimus rusticus	16.93	22.75
	Macquartia praefica	8.64	4.42
	Meigenia dorsalis	6.19	1.97
	Meigenia unicata	6.58	2.28
	Melanagromyza chaerophylli	1.65	0.08
	Melangyna labiatarum	8.5	4.25
	Melanogaster nuda	5.95	1.79
	Melanomya nana	4.29	0.81
	Melanostoma mellinum agg.	6.51	2.23
	Melanostoma scalare	8.1	3.78
	Melinda gentilis	6.18	1.96
	Melinda viridicyanea	8.28	3.99
	Meliscaeva cinctella	10.63	7.32
	Merodon equestris	15.88	19.46
	Meromyza femorata	4.64	0.98
	Mesembrina meridiana	12.46	10.77
	Microchrysa flavicornis	4.74	1.03
	Microsoma exiguum	4.44	0.88
	Morellia aenescens	7.16	2.8
	Morellia hortorum	7.78	3.43
	Morellia podagrica	7.94	3.6
	Morellia simplex	7.07	2.72
	Musca autumnalis	5.74	1.64
	Musca domestica	7.06	2.71
	Musca osiris	5.43	1.43
	Muscina levida	7.47	3.11
	Muscina pascuorum	9.53	5.61
	Muscina prolapsa	8	3.67
	Myathropa florea	14.96	16.82
	Mydaea nebulosa	7.25	2.89
	Myospila meditabunda	6.79	2.47
	Neomyia cornicina	6.99	2.64

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Neomyia viridescens*	8.59	4.36
	Nephrotoma appendiculata	14.88	16.6
	Nephrotoma flavescens	10.58	7.24
	Nilea rufiscutellaris	8.84	4.68
	Nowickia ferox	14.36	15.22
	Nyctia halterata	6.59	2.29
	Ocytata pallipes	7.97	3.64
	Onesia floralis	8.7	4.5
	Opesia cana	8	3.67
	Ophiomyia nasuta	2.57	0.24
	Opomyza germinationis	5.47	1.46
	Orellia falcata	7.13	2.78
	Oscinella frit	2.38	0.2
	Oscinella nigerrima [5)]	1.75	0.09
	Oxyna flavipennis	5.97	1.8
	Pales pavida	8.55	4.31
	Paracraspedothrix montivaga	3.19	0.4
	Paragus haemorrhous	5.55	1.51
	Paramacronychia flavipalpis	11.92	9.67
	Parasyrphus annulatus	8.11	3.79
	Pegomya meridiana	3.33	0.44
	Pegoplata aestiva	5.13	1.25
	Pegoplata infirma	4.1	0.73
	Pegoplata nigroscutellata	5.35	1.38
	Pelecocera tricincta	5.29	1.35
	Phaonia angelicae	8.31	4.02
	Phaonia consobrina	9.41	5.44
	Phaonia meigeni	8.68	4.47
	Phaonia pallida	7.15	2.79
	Phaonia rufiventris	7.5	3.14
	Phaonia serva	9.72	5.89
	Phasia hemiptera	10.96	7.88
	Phasia obesa	5.93	1.78
	Phasia pusilla	4.35	0.84
	Pherbellia cinerella	5.04	1.2
	Pherbellia griseola	3.8	0.61
	Phorbia bartaki	4.26	0.8
	Phorbia genitalis	4.38	0.85
	Phorbia juncorum	4.49	0.91
	Phorbia unipila	4.85	1.09
	Phryxe heraclei	7.75	3.4
	Physiphora alceae	5.44	1.44
	Phytomyza albipennis	3.29	0.43
	Pipiza noctiluca	9.52	5.6
	Pipiza quadrimaculata	7.4	3.04
	Pipizella viduata	6.02	1.84
	Platycheirus albimanus	7.24	2.88
	Platycheirus clypeatus	7.94	3.6
	Platycheirus europaeus	8.86	4.7
	Platycheirus manicatus	9.59	5.7
	Platycheirus peltatus	9.75	5.93
	Platycheirus tarsalis	7.8	3.45
	Platymya fimbriata	6.53	2.24
	Platypalpus agilis	3.74	0.58
	Platypalpus albiseta	3.15	0.39
	Platypalpus cruralis	5.83	1.7
	Platystoma seminationis	5.71	1.62
	Polietes lardarius	8.88	4.73
	Pollenia amentaria	10.03	6.36
	Pollenia angustigena	7.58	3.22
	Pollenia hungarica	7.17	2.81
	Pollenia labialis	8.48	4.23
	Pollenia pediculata	7.15	2.79
	Pollenia rudis	6.84	2.51
	Prosena siberita	5.19	1.29

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Protocalliphora falcozi*	9.8	6.01
	Protocalliphora peusi	10.54	7.17
	Protophormia terraenovae [6)]	8.5	4.25
	Psila merdaria [7)]	8.4	4.13
	Pyrellia rapax	11.78	9.4
	Ravinia pernix	9.89	6.14
	Rhagio scolopaceus	10.32	6.81
	Rhagio strigosus	8.99	4.87
	Rhamphomyia longipes	10.81	7.62
	Rhamphomyia sulcata	13.03	12.01
	Rhingia campestris	7.78	3.43
	Rhinomorinia sarcophagina	5.12	1.24
	Saltella sphondylii	4.38	0.85
	Sarcophaga aratrix	10.32	6.81
	Sarcophaga bulgarica	7.48	3.12
	Sarcophaga caerulescens	7.42	3.06
	Sarcophaga carnaria	5.35	1.38
	Sarcophaga crassimargo	8.79	4.61
	Sarcophaga depressifrons	7.93	3.59
	Sarcophaga dissimilis	2.9	0.32
	Sarcophaga incisilobata	15.38	18
	Sarcophaga jeanleclercqi	10.55	7.19
	Sarcophaga melanura	7.15	2.79
	Sarcophaga nemoralis	6.84	2.51
	Sarcophaga noverca	8.43	4.17
	Sarcophaga pumila	10.7	7.44
	Sarcophaga schineri	9.85	6.08
	Sarcophaga subvicina	7.48	3.12
	Sarcophaga vagans	10.14	6.53
	Sarcophaga variegeta	5.74	1.64
	Sarcophaga vivina	9.47	5.53
	Scaeva pyrastri	13.02	11.99
	Scaeva selenitica	14.73	16.2
	Scaptomyza graminum	2.18	0.16
	Scathophaga stercoraria	9.14	5.07
	Scatopsciara sp.	NA	NA
	Schwenckfeldina carbonaria	3.98	0.68
	Sciara flavimana	3.88	0.64
	Sepsis cynipsea	3.86	0.63
	Sepsis duplicata	2.33	0.19
	Sepsis thoracica	3.58	0.52
	Sicus ferrugineus	10.12	6.49
	Siphona geniculata	5.69	1.61
	Siphonella oscinina	7.25	2.89
	Solieria pacifica	7.76	3.41
	Sphaerophoria interrupta group	7.25	2.89
	Sphaerophoria scripta	8.53	4.29
	Sphaerophoria taeniata	9.2	5.15
	Strongygaster globula	6.16	1.95
	Synathica parvula	5.21	1.3
	Syritta pipiens	3.82	0.61
	Syrphus ribesii	8.88	4.73
	Syrphus torvus	10.63	7.32
	Syrphus vitripennis	10.75	7.52
	Tachina fera	10.89	7.76
	Tachina magnicornis	11.93	9.69
	Tephritis crepidis	4.25	0.79
	Thecophora atra	7.01	2.66
	Thecophora cinerascens [8)]	4.5	0.91
	Thecophora distincta	5.96	1.8
	Thecophora fulvipes	5.23	1.31
	Thereva valida	11.18	8.28
	Thricops cunctans	6.29	2.05
	Thricops longipes	8.59	4.36
	Thricops nigrifrons	7.4	3.04

order	species	body size [mm]	predicted body mass [mg]
Diptera	*Thricops semicinereus*	5.8	1.68
	Tipula vernalis	19.17	30.81
	Tolmerus atricapillus	15.16	17.38
	Toxoneura quinquemaculata	5.33	1.37
	Trachysiphonella ruficeps [9)]	1.5	0.06
	Trachysiphonella scutellata	2.68	0.26
	Trichactia pictiventris	6.14	1.93
	Trixa conspersa	10.66	7.37
	Volucella bombylans	16.87	22.55
	Volucella pellucens	16.99	22.94
	Voria ruralis	7.8	3.45
	Winthemia quadripustulata	9.7	5.86
	Xanthogramma pedissequum	11.65	9.15
Hymenoptera	*Andrena agilissima*	16.96	30.74
	Andrena anthrisci	7.63	4.53
	Andrena bicolor	10.95	10.67
	Andrena carantonica	13.61	18.01
	Andrena chrysosceles	10.02	8.63
	Andrena cineraria	13.23	16.82
	Andrena combinata	10.88	10.51
	Andrena dorsata	9.57	7.74
	Andrena flavipes	11.63	12.33
	Andrena florivaga	10.14	8.88
	Andrena fucata	10.64	9.97
	Andrena fulva	13.75	18.47
	Andrena fulvago	8.31	5.54
	Andrena gravida	14.01	19.32
	Andrena haemorrhoa	11.38	11.71
	Andrena minutula	6.43	3.03
	Andrena nigroaenaea	13.95	19.12
	Andrena nitida	14.74	21.85
	Andrena nitiduscula	8.65	6.09
	Andrena potentillae	7.89	4.9
	Andrena proxima	10.34	9.31
	Andrena semilaevis	8.23	5.41
	Andrena subopaca	6.79	3.44
	Andrena tibialis	13.58	17.92
	Andrena varians	12.11	13.59
	Apis mellifera	13.54	17.79
	Arge rustica	7.05	3.76
	Athalia rosae	7.05	3.76
	Bombus bohemicus	20.82	50.79
	Bombus hortorum	18.41	37.56
	Bombus humilis	13.02	16.19
	Bombus lapidarius	17.01	30.96
	Bombus lucorum agg.	14.46	20.86
	Bombus pascuorum	18.81	39.59
	Bombus pratorum	11.73	12.59
	Bombus rupestris	22.74	63.1
	Bombus soroeensis	16.94	30.65
	Bombus subterraneus	21.8	56.87
	Bombus sylvarum	12.87	15.74
	Bombus terrestris	13.18	16.67
	Bombus vestalis	20.3	47.73
	Bombus wurflenii	16.27	27.78
	Brachymeria sp.	NA	NA
	Cephus pygmeus	9.52	7.64
	Chelostoma florisomne[9)]	5.5	2.11
	Formica cunicularia	4.65	1.43
	Formica lusatica	7	3.7
	Halictus eurygnathus	10.6	9.88
	Halictus rubicundus	11.54	12.11
	Halictus simplex	9.78	8.15
	Halictus tumulorum	6.66	3.29
	Hylaeus annularis	5.98	2.56

order	species	body size [mm]	predicted body mass [mg]
Hymenoptera	*Lasioglossum albipes*	8.44	5.74
	Lasioglossum calceatum	9.96	8.51
	Lasioglossum fulvicorne	6.93	3.61
	Lasioglossum laevigatum	8.04	5.12
	Lasioglossum laticeps	6.63	3.25
	Lasioglossum lativentre	6.94	3.62
	Lasioglossum leucopus	5.42	2.04
	Lasioglossum leucozonium	9.45	7.51
	Lasioglossum lineare	6.73	3.37
	Lasioglossum minutulum	5.16	1.82
	Lasioglossum morio	5.01	1.7
	Lasioglossum pauxillum	6.02	2.6
	Lasioglossum villosulum	6.35	2.94
	Lasioglossum zonulum	8.45	5.76
	Lasius emarginatus	3.11	0.57
	Lasius niger	3.98	1
	Melitta haemorrhoidalis	10.4	9.44
	Myrmica rubra	4.63	1.42
	Myrmica ruginodis	4.45	1.29
	Nomada flavoguttata	5.61	2.21
	Osmia bicornis	10.53	9.72
	Selandrina serva	6.44	3.04
	Sphecodes ephippius	7.52	4.37
	Sphecodes ferruginatus	7.62	4.51
	Sphecodes hyalinatus	5.79	2.37
	Tapinoma ambiguum	14.23	20.06
	Temnothorax saxonicus	3.28	0.65
	Tenthredo atra	12.81	15.56
	Tenthredo notha	10.07	8.74
	Tenthredo temula	12.31	14.14
	Tenthredo vespa	11.16	11.17
	Tiphia femorata	8.19	5.35
	Trachusa byssinum	10.8	10.33
Lepidoptera	*Adscita statices*	26.5	147.32
	Aglais io	21.15	76.02
	Aglais urticae	17.7	45.12
	Aphantopus hyperantus	14.05	22.96
	Coenonympha glycerion	10.11	8.79
	Cupido minimus	8.4	5.12
	Cyaniris semiargus [10)]	13.5	20.43
	Erebia aethiops	14.37	24.52
	Erynnis tages [10)]	24.5	117.01
	Hesperia comma	17.34	42.49
	Maniola jurtina	15.35	29.74
	Melanargia galathea	16.11	34.26
	Melitaea aurelia [10)]	12.35	15.76
	Nymphalis polychloros [10)]	52.5	1102.31
	Ochlodes sylvanus [10)]	14.8	26.73
	Papilio machaon	25.6	133.11
	Pieris brassicae	21.9	84.19
	Pieris napi	16.26	35.2
	Pieris rapae	17.02	40.23
	Plebeius argus/argyrognomon/ideas agg.	12.3	15.57
	Polyommatus bellargus	12.43	16.06
	Polyommatus coridon	13.79	21.74
	Polyommatus icarus	11.28	12.1
	Thymelicus lineola	13.98	22.63
	Thymelicus sylvestris	14.88	27.16
	Vanssa cardui	20.3	67.41
	Zygaena carniolica	13.16	18.97
	Zygaena filipendulae	17.03	40.3

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CHAPTER III

Surface area – volume ratio in pollinating insects

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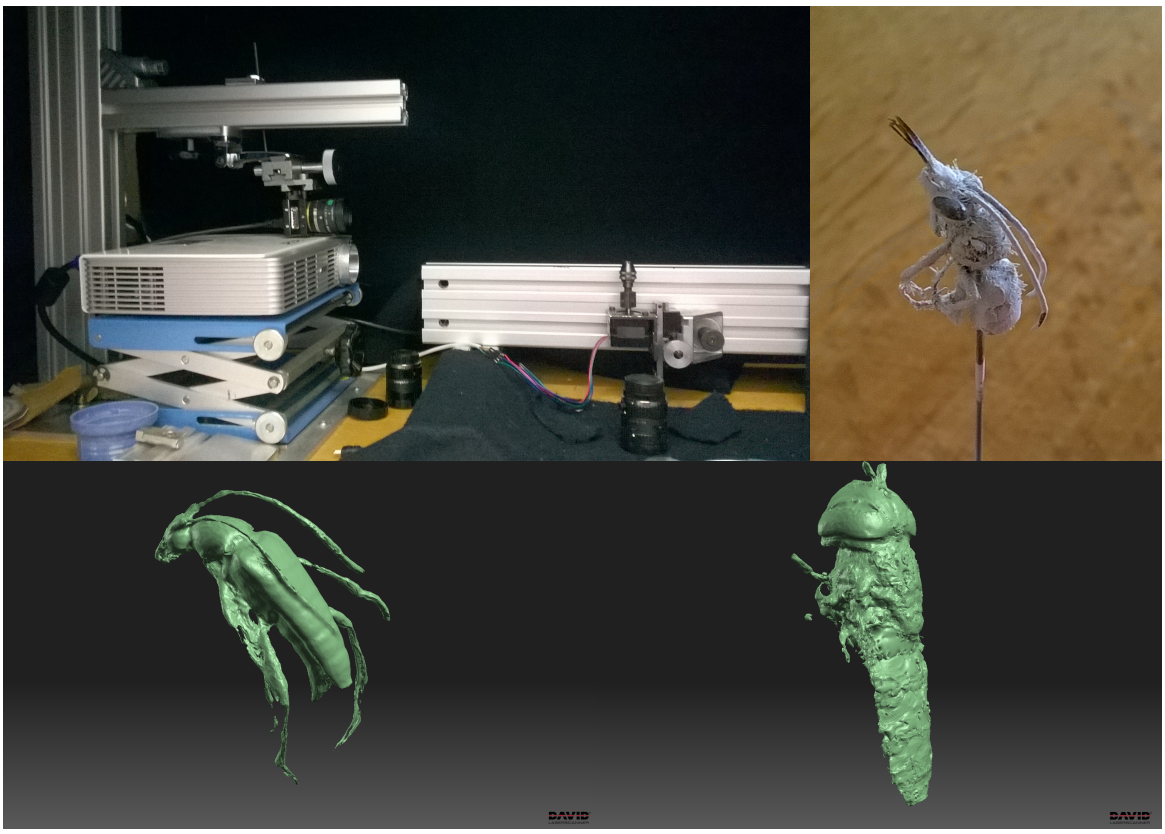

Abstract

Body mass, volume and surface area are important for many aspects of the physiology and performance of species. Whereas body mass allometries received a lot of attention in the literature, surface areas of animals have not been measured explicitly in this context. We quantified surface area – volume (*SA/V*) ratios for the first time using 3D surface models of insects as obtained by structured light scanning methods. Water loss of 67 pollinator species was measured gravimetrically at extremely dry conditions for two hours at 15° and 30°C to demonstrate the applicability this new method and relevance for predicting the performance of insects. Quantified *SA/V* ratios significantly explained the variation in water loss across species, both directly or after correcting for the prediction of body mass allometry ($SA/V \sim \text{mass}^{2/3}$). Small insects with a proportionally larger surface area had the highest water loss rates, a consistent trend within each of the four orders (Diptera, Hymenoptera, Lepidoptera and Coleoptera) that did not differ significantly in water loss. Directly measured *SA/V* ratios thus provide a promising method to predict physiological responses of insects, improving the potential of predicted relative changes of *SA/V* based on body mass allometry alone.

KEYWORDS: body size, body shape, 3D surface imaging, sphericity, structured light, water loss

Introduction

Biodiversity Based on present evidence, body size is considered as the most important trait of an animal species (Brown 1995). Body size affects many aspects of insect ecology and physiology, including water loss rates (Le Lagadec *et al.* 1998) and heat balance (Bartholomew & Casey 1978, Stone 1993). One parameter that shows a fundamental change with body size is the animal's surface area. Since the surface represents the interface between the animal and its environment, e.g. for exchange of water, temperature as well as infections (e.g. Porter & Gates 1969), surface areas may be one of the main drivers of the variation in responses of animals. Most terrestrial insects are very small with body lengths typically between 1 and 20 mm, and consequently – for a given shape – have higher surface area – volume (*SA/V*) ratios compared to larger animals. Traditionally, surface areas have been predicted by such allometric scaling laws based on body mass rather than measured directly, since quantitative measurements of complex surfaces are inherently difficult to perform (Porter & Gates 1969, Lighton & Feener 1989). Here we used a modified structured light scanner to create 3D-models of insects (after Winkelbach *et al.* 2006), allowing us to quantify surfaces and volumes of different species and thereby to separate effects of body mass from effects of body shape and surface area. This

will help to explain different responses of animals with equal size to a given environmental condition. In our study on pollinating insects, we expect that water loss rates relative to the body water content decrease with the species' body mass, but we would like to test whether this pattern is driven by body size itself or by variation in surface area.

Water loss characteristics in general are strongly related to body size (Gibbs 2003, Chown & Klok 2003). A larger body size, often defined as body mass, represents an advantage in terms of a reduced water loss rate. A large surface area could be critical for water loss of insects, since total evaporation is a function of surface area (Hadley 1994). However, body shapes of insects are complex, and their surface areas have not been adequately quantified so far in the context of water loss or other physiological responses. The relevance of body size to water loss suggests that small animals are more likely affected by water saturation deficits, especially at high temperatures and increasing variation in rainfall (Fung *et al.* 2011, New *et al.* 2011, Sanderson 2011). Changes in water availability due to rising average temperatures and higher frequencies of extreme weather events such as longer dry periods, can have severe consequences for species communities (Jentsch *et al.* 2009). A higher temperature implies more short-term drought stress for insects due to increasing water loss (Hadley 1970, Gibbs 2011). Thus, the availability of water becomes an important factor, particularly for small animals such as insects (Chown & Nicolson 2004). Responses to water availability have a pronounced effect on the insects' activities, distribution ranges and their species diversity (Hawkins *et al.* 2003). Short-term drought stress of pollinators could also affect pollination of crops and wild plants if insects with high water loss avoid too warm conditions.

The ability of insects to tolerate variation in water content is highly variable (Wigglesworth 1945). Previous studies, that compare water loss of different taxa only focused on respiratory water loss during gas exchange (Woods & Smith 2010, Chown 2002). Therefore, we investigated the interspecific variability between various pollinators in terms of relative water loss and body water content.

Water loss occurs due to evaporation through the cuticle and by respiration. Cuticular water losses usually represent more than 80% of the total water loss in many taxa at normal conditions (Chown 2002). Thus, most of the water is lost over the cuticular surface, although epicuticular waxes limit such losses and although the quality and quantity of cuticular hydrocarbons vary between taxa and influence the evaporation (Gibbs 2002).

We thus quantified the surface and volume for different insect species that pollinate flowers, and test whether the interspecific variation in relative water loss can be explained by the *SA/V* ratio also after

correcting for the body size alone. We studied water losses at two different temperatures and standardized for very low moisture conditions, and tested whether taxonomic groups and water content influenced the water loss rates.

Material and Methods

Pollinator sampling

Pollinators were collected between May and August 2013 and in June 2014 on grassland sites in the National Park Hainich and the surrounding area in central Germany and in Darmstadt in southwestern Germany. Grassland sites in the Hainich region (51.1° N, 10.4° E) were two flower rich meadows that are part of the Biodiversity Exploratories Project (Fischer *et al.* 2010). Grassland sites were surrounded by cereal fields, forest and further grasslands. The two study sites in Darmstadt were meadows around the Campus Lichtwiese and in the Botanical Garden of the Technical University (49.9° N, 8.7° E). These sites were surrounded by hedges and urban structures.

Insects sitting on flowers were captured with a dip net and transferred into a glass sealed by foam. Since all insects were caught on flowers and are known at least on genus level as potential pollinators, they are summarized as 'pollinators' in this study.

Before the subsequent measurements, the animals were kept in the vessels and a moist cellulose cloth was added in order to ensure the supply of water for the insects and not to induce short-term drought stress before the beginning of water loss measurements. Insects were stored at 15°C. The measurements of water loss were carried out either directly after sampling or up to 3 days later. We could not control for hydration status of insects, as they were captured in the field. We furthermore, could not directly select specific species as most pollinators could only be identified after the experiments by specialized taxonomists.

3D-measurements

A modified structured light scanner (based on the SLS1-system, David Vision Systems GmbH, Koblenz, Germany; Winkelbach *et al.* 2006, see also Zhang & Wei 2002, Geng 2011) was used to generate 3D-surface models of insects. The resulting surfaces were used to measure surface area and volume in Amira® 5.6 (FEI, Munich, Germany). Modifications include elongation of the optical tube of the

beamer to allow for short-distance focus and usage of a step-motor (controlled by an arduino based hardware-software-interface; see http://www.arduino.cc) to automatically generate images from several (at least 20) different angles until an automatic fusion of single projections was possible. The SLS-system was calibrated using custom-made small templates based on the scale-templates provided by the manufacturer. The wings of the insects were removed because they could not be pictured with the surface scans. In order to achieve an optimal result for the surface reconstruction and also to be able to well resolve fine structures, all pollinators were coated with a matte nanoparticle coating spray of David Vision Systems GmbH before scanning. To test the reproducibility of the method for measuring the surface area (*SA*) and volume (*V*), one individual honeybee (*Apis mellifera,* L.) was scanned repeatedly with the same parameter settings – the measured surface varied less than 2% and the volume varied 3% (*SA*: 223.6 ± 3.6 mm^2, *V*: 164.9 ± 5.1 mm^2, mean ± SD, $N = 5$). Also, results of high-resolution measurements done with synchrotron radiation (*Coccinella septumpunctata,* L., *SA*: 118.3 mm^2, *V*: 59 mm^3, method see (Heethoff et al., 2008, Betz et al., 2007) hardly differed from those of the structured light scanner (*SA*: 118.6 mm^2, *V*: 65 mm^3) (Fig. 3.1). To explore the variation within a species, we scanned 7 individual bumblebees (*Bombus lapidarius,* L.) and 5 hoverflies (*Episyrphus balteatus,* Deg.). As expected, although their *SA* and *V* were relatively variable (*B. lapidarius SA*: 3.62 ± 0.89 mm^2, *V*: 214.10 ± 53.34 mm^3; *E. balteatus SA*: 0.98 ± 0.13 mm^2, *V*: 34.71 ± 6.69 mm^3), their shapes and thus *SA/V* ratios were quite constant (*B. lapidarius:* 0.017 ± 0.001 mm^{-1}; *E. balteatus:* 0.028 ± 0.002 mm^{-1}). Hence, the SLS-system produced reliable and reproducible results, and measurements from a single individual were thus used to characterize the shape of an insect species.

Twenty-one species in the analyses were represented by more than one individual (2 – 5). We scanned only one individual per species and extrapolated the surface and volume of other individuals based on their dry mass (*m*), assuming a constant specific mass ($V \sim m$) and shape ($SA \sim m^{2/3}$) (see Fig. 3.1). Volume was obtained as $V = a_V \times m$ and $SA = a_S \times m^{2/3}$, where a_V and a_S were obtained from the scanned individual. For the two species above with 5-7 individuals each, observed and extrapolated surface areas (where surfaces of all but a single individual were extrapolated) were highly linearly correlated (*B. lapidarius*: $r^2 = 0.99$; *E. balteatus*: $r^2 = 0.91$). The SLS-method works best for smooth surfaces, while it has some problems with dense hairs or bristles that may inflate the *SA* (see Fig. 3.1 bottom and outlier in Fig. 3.2).

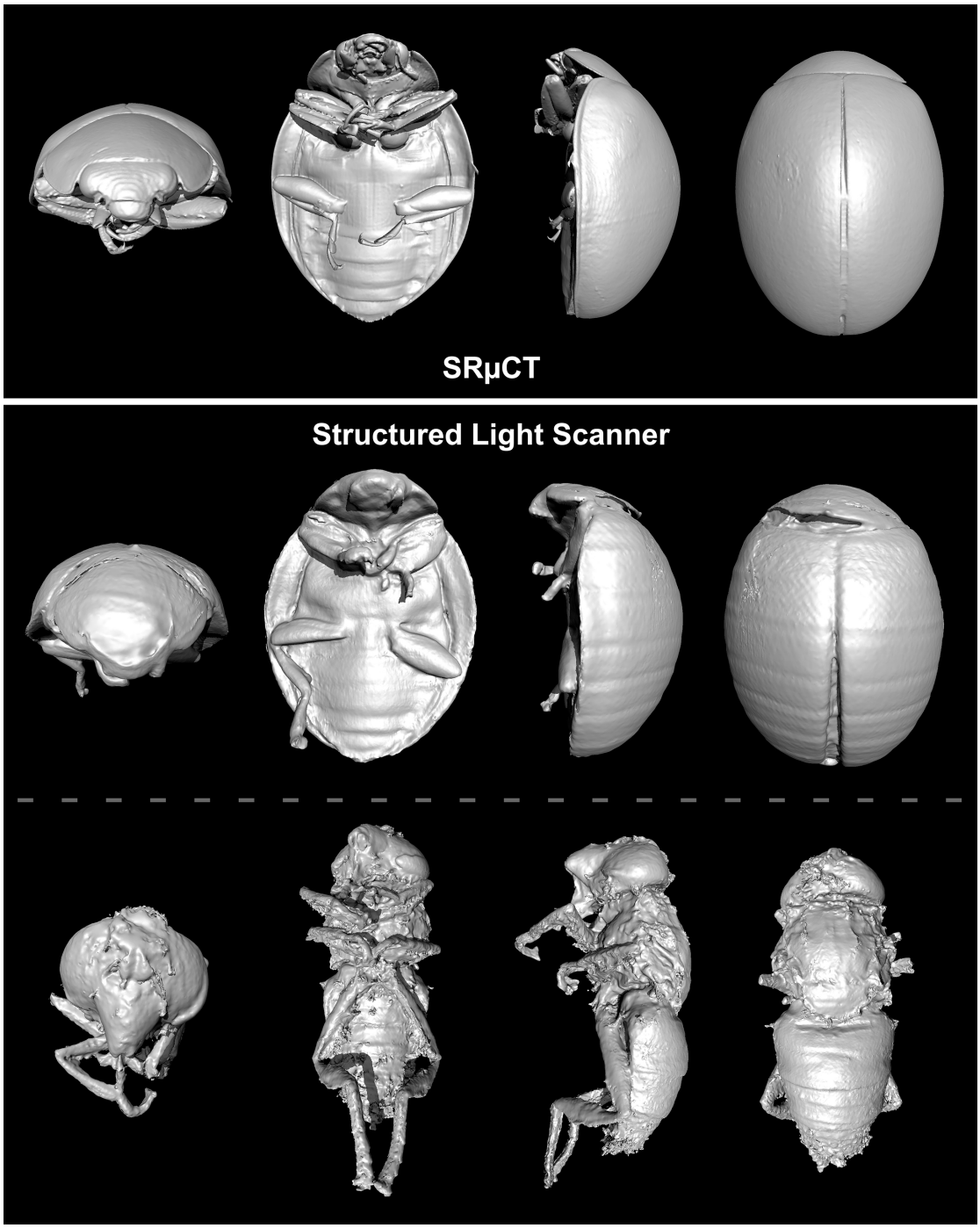

Fig. 3.1: Real 3D-surface model of ladybug (*Coccinella septumpunctata*; Coleoptera) measured with Synchrotron μCT compared to models from structured light scanner of *C. septumpunctata* (same individual) and that of a hoverfly (*Eristalis tenax*; Syrphidae).

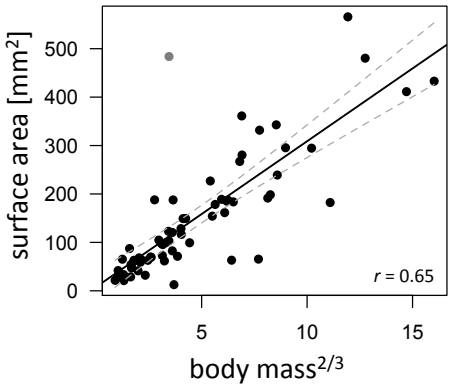

Fig. 3.2: Linear regression of measured surface area and via body mass predicted surface area. Dotted lines represent 95% confidence intervals. The grey outlier represents a small fly (*Sarcophaga haemorrhoa*) with many erect bristles that contribute to a very high projected surface area.

Water loss measurements

Measurements of water loss were performed according to the gravimetric method described by (Hadley 1994). Insects that obviously excreted urine or feces during the measurements were excluded from the analysis so that water loss could be assumed to be equivalent to mass loss (Wharton & Richards 1978). For water loss measurements, the insects were transferred in small cloth bags (about 5 × 5 cm), which were composed of an organza fabric, a synthetic polyamide fiber, and closed with adhesive tape. The bags were placed in glass vials, tightly sealed with a plastic lid (80 × 25 × 25 mm). For the measurement of water loss climatic chambers were set to two different air temperatures (15°C and 30°C, thus representing moderate and high temperature conditions) to simulate conditions of different short- term drought stress. To maintain constant dry conditions, calcium chloride ($CaCl_2$) was added into the vials as a drying agent, which led to an air humidity level of 5 % inside the vials (thus unnatural, stressful conditions). To avoid contact with the specimen, the coarse powder was filled into micro tubes that were previously punctured with a needle. We did not vary humidity conditions, because we assume that species rankings in water loss are relatively similar irrespective of the humidity level (Hood & Tschinkel, 1990). Each treatment lasted 2 hours. The conditions in the vials during the 2h measurements were monitored with temperature and humidity sensors (iButtons®, Measurement Systems Ltd, United Kingdom). The relative humidity inside the vials containing the insects has been effectively reduced to an average of about 5% relative humidity at 15°C (mean ± standard deviation: 5.4 ± 0.5, N= 25) and at 30°C (4.7 ± 0.3, N = 25, measurement intervals: 5 min.). Temperatures in these vials during the measurements also remained constant (14.9 ± 0.3

versus 29.2 ± 1.5). Lights within the climatic chambers were turned off during the experiments to minimize uncontrolled variation in insect activity; no nocturnal species were involved.

Body mass of each insect was determined with a microbalance (Mettler Toledo, XS3DU, readability: 0.1 g) four times: (a) the initial fresh mass of the insect m_1 prior to the dryness experiment, (b) its mass after the first dryness treatment m_2 (15°C, 2h), (c) and after the second dryness treatment m_3 (30°C, 2h), and finally (d) its dry mass m_{dry} after it has been dried until weight constancy in an oven at 60°C for at least 4 days. Insects remained in their bags from $m_1 - m_3$, hence the weight of the bags was subtracted. The weight of the bags before the experiment was determined after their acclimation to room conditions. Not only the insects, but also the bags lost water during the experiments. We quantified this with a series of measurements with empty bags. At 15°C bags lost a mean of 1.25% (± 0.041 SE, $N = 63$) of their weight, and at 30°C 2.02% (± 0.061, $N = 62$). Weighing of animals at the end of the measurements took about 10 min. The insects were transported to the microbalance in a desiccator to prevent weight changes caused by ambient air humidity. After each experiment we checked whether the insects were still alive. Dead insects were removed from the analysis. The insects were subsequently identified to species level.

The main response variable in our study is the relative water loss at 15°C and 30°C, defined as proportional loss of the water body before each experiment:

$WL_{15°C}$ [%] $= (m_2 - m_1) / (m_1 - m_{dry}) \times 100\%$ and

$WL_{30°C}$ [%] $= (m_3 - m_2) / (m_2 - m_{dry}) \times 100\%$.

We also calculated the water content of the insects as

$WC = (m_1 - m_{dry})/m_{dry} \times 100\%$.

Given the sequential experiments for each individual (30°C following the 15°C treatment), the amount of water loss at 30°C is not directly comparable with 15°C, since responses are not linear over time but saturate with the length of dry conditions (Hadley 1994). Nevertheless, for each species, water loss (WL) at 15°C corresponded very well to its water loss at 30°C (linear regression $r^2 = 0.95$, $p < 0.001$, $WL_{30°C} = 0.98 \times WL_{15°C} + 11.72$, $N = 67$ spp.). Differences between $WL_{30°C}$ and $WL_{15°C}$ were independent of body size ($r^2 < 0.01$, $p = 0.824$).

Statistics

Water loss of pollinators was measured for 99 individuals (67 species). Where different individuals were collected from the same species, we used the mean water content, water loss, dry mass (as a measure for body size) and *SA/V* ratio to remove replication within a species that could bias across-species comparisons. For many species we did not have replicated individuals, since we tried to maximize the taxonomic breadth of represented pollinators, and since water loss measurements were performed without prior taxonomic identification, which was carried out by different specialists. The two regions have been pooled for analysis because only three species occurred in both. We assessed main and interaction effects of the predictors body mass and order on the response variable water content with linear mixed models. The number of days until the measurement was employed as random factor to control for a potential bias water loss during the storage, but the general results remained unchanged when this random factor was removed. Water loss (*WL*) and body mass (m) were log transformed where appropriate (but not m in non-linear regressions with m^b) to meet the assumptions of constant error variance and normality of errors. In addition we assessed main and interaction effects of *SA/V* ratio and order on water loss at 15°C and 30°C with the same method. Effects of water content and *SA/V* ratio are shown for taxonomic groups of insect orders. Hoverflies and bumblebees were separated from flies and hymenopterans, respectively, as hoverflies are commonly used as bioindicator (Biesmeijer et al., 2006), and bumblebees strongly differ in their size, morphology and physiology from other hymenopterans (Heinrich, 1975).

As another potential predictor of water loss, we calculated sphericity (ψ) of pollinators, a characteristic of the compactness of an object:

$$\psi = \left(\pi^{1/3} \cdot 6^{2/3}\right)/a,$$

where a is the slope of the surface area to volume relationship (see Fig. 3.2). Sphericity (ψ) equals 1 for a sphere and $0 < \psi < 1$ for any other shape, e.g. $\psi = 0.806$ for a cube. Note that whereas *SA/V* scales allometrically with V or m for a given shape, a and thus is constant irrespective of the level of V and m. Statistics were conducted in R 2.15.1 (R Development Core Team 2012) with the 'nlme' package (Pinheiro et al., 2011).

Results

We measured surface area (*SA*) and volume (*V*) of 67 pollinator species belonging to the orders Diptera (25 species; including 8 syrphids), Coleoptera (15), Lepidoptera (14) and Hymenoptera (13, incl. 3 bumblebees). Generally, body mass – namely the predicted surface area based on body mass allometry ($m^{2/3}$) used in previous studies –explained 65% of the variation of measured surface area ($F = 122.2$, $p < 0.001$, Fig. 3.2). Volume of the insects was linearly related to body mass ($\log(V) \sim \log(m)$, $r^2 = 0.89$, $P < 0.001$). Their surface area increased with a 2/3 power term over volume ($SA \sim V^{2/3}$, $r^2 = 0.49$, $p < 0.001$), and furthermore, surface area to volume (*SA/V*) ratio decreased over volume ($SA/V \sim V^{-1/3}$, $r^2 = 0.46$, $p < 0.001$) (Fig. 3.3a). While these geometric relationships generally hold true for any object, their slopes are specific for each shape, ranging from spheres to more complex shapes. As expected, beetles have the lowest slope of the four orders, while the slope of the other pollinators was higher but very similar (Fig. 3.3b). These slopes can be standardized as sphericity (ψ, supplementary Table 3.1).

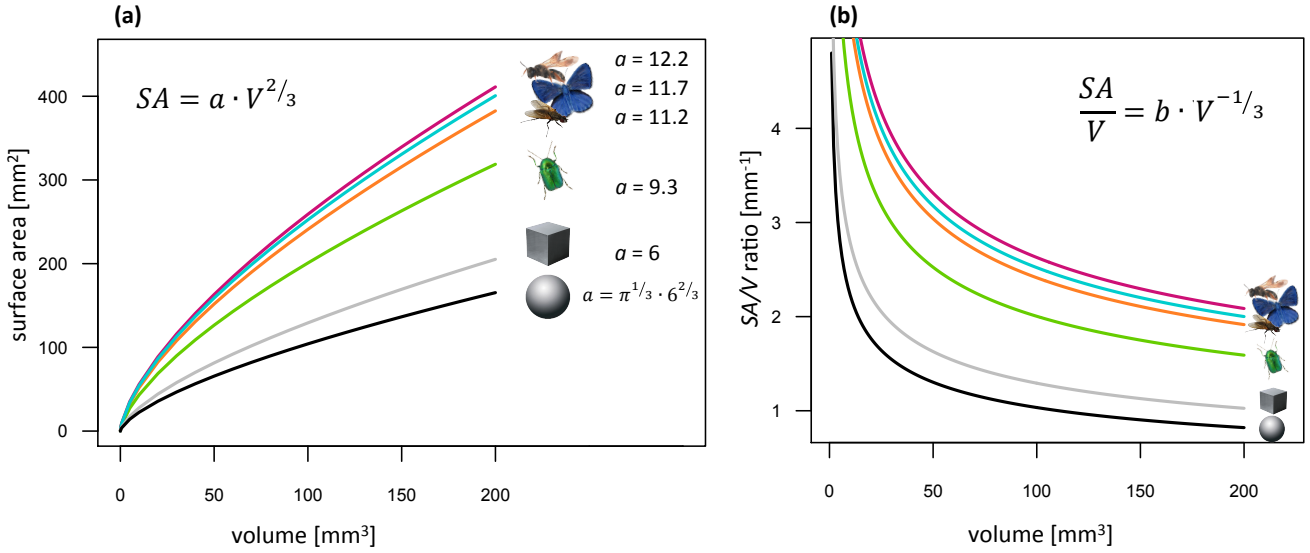

Fig. 3.3: Relationships between surface area (SA) and volume (V). **(a)** SA/V ratio over V and **(b)** SA over V for defined geometric bodies and mean shape of the four insect orders; from bottom to top: sphere, cube, beetles, flies, butterflies and hymenopterans. The surface area SA increases at a saturating rate (power term 2/3) with volume V for any object, hence its SA/V ratio decreases with V (or mass m if V ~ m). The slopes of these relationships **(a, b)** differ with the shape of the object.

At both 15° and 30°C water loss rates (relative to water body content) were significantly predicted by *SA/V* ratios, whereas taxonomic orders did not differ significantly (Table 3.1). However, there was a significant (at 15°C) or marginally significant (at 30°C) interaction between *SA/V* ratio and order, indicating that the effect of *SA/V* ratio was not consistent in all orders. In beetles, non-syrphid flies and other hymenopterans, *SA/V* ratio had a significant effect on water loss at 30°C (Fig. 3.4c), but not in

bumblebees, butterflies and hoverflies. *SA/V* ratio alone explained 37 % of the variation in water loss WL ($WL_{15°C} \sim SA/V$, $p < 0.001$) at 15°C and 34% at 30°C ($p < 0.001$).

Table 3.1: Effects of body mass, surface area to volume ratio and insect order on water content and relative water loss referred to water body (at 15°C and 30°) of pollinators. Predictors with $p < 0.05$ were considered to be significant (in bold).

	df_{num}	df_{den}	F	P
water content [%]				
body mass	1	52	33.3	**< 0.001**
order[1])	3	52	4.8	**0.005**
body mass × order	3	52	2.6	0.065
water loss at 15°C [%]				
SA/*V* ratio	1	52	58.3	**< 0.001**
order	3	52	2.0	0.127
SA/*V* ratio × order	3	52	3.3	**0.027**
water loss at 30°C [%]				
SA/*V* ratio	1	52	56.6	**< 0.001**
order	3	52	2.1	0.107
SA/*V* ratio × order	3	52	2.8	0.051

[1)] Tukey HSD: hymenopterans – flies ($p = 0.001$), lepidopterans – flies ($p = 0.022$), all others $p \geq 0.14$).

Comparing the different insect orders, relative water loss at 15°C ranged between 20% (butterflies) and 34% (flies). At 30°C, pollinators lost between 30% (butterflies) and 46% (flies) of their water body (i.e. fresh mass – dry mass). Since variation across species within the orders was high, no significant differences were found (Fig. 3.4a/b). Water content of pollinators varied systematically with body size and across taxa. It was between 69% (hymenopterans) and 74% (flies) and decreased with increasing body mass (Table 3.1, supplementary Table 3.2. Hymenopterans and butterflies had significantly lower water content than flies, but water content of beetles did not differ significantly from the other orders (Fig. 3.4d). There was no significant interaction between body mass and insect order, indicating that the effect of body mass on water content was similar in all groups. Effects of six taxonomic groups were similar as those found for the four orders (supplementary Table 3.2, 3.3).

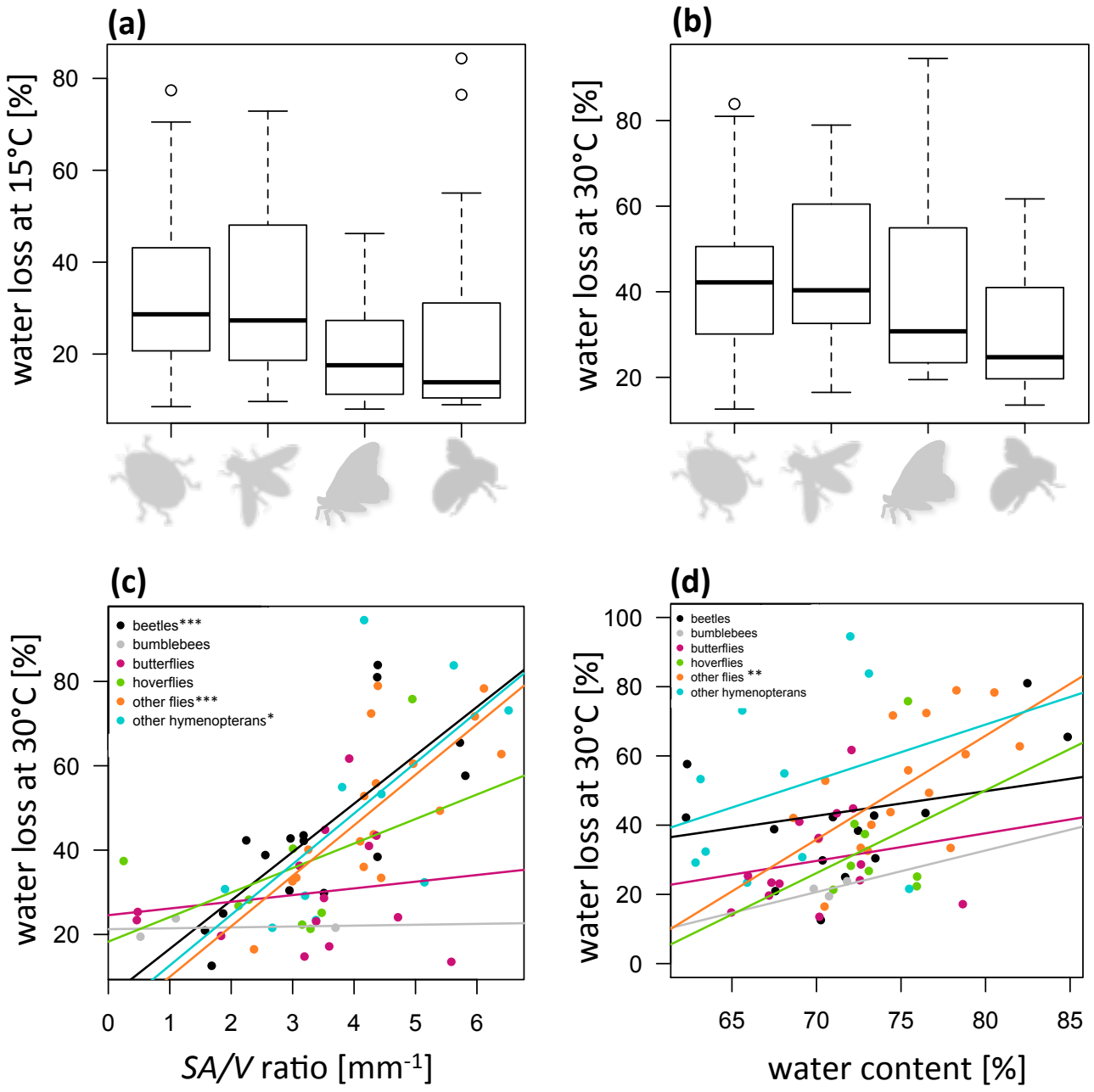

Fig. 3.4: Relative water loss (based on water body) of pollinator orders at 15°C **(a)** and 30°C **(b)**. From left to right: beetles, flies, butterflies and hymenopterans. Effects of surface area to volume ratio **(c)** and water content **(d)** on water loss at 30°C of pollinator groups. Here we split dipterans and hymenopterans into two taxa (see supplementary Table 3.2). Results are from linear regression models. Significances: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Water content of pollinators varied systematically with body size and across taxa. It was between 69% (hymenopterans) and 74% (flies) and decreased with increasing body mass (Table 3.1). Hymenopterans and butterflies had significantly lower water content than flies, but water content of beetles did not differ significantly from the other orders (Fig. 3.4d). There was no significant interaction between body mass and insect order, indicating that the effect of body mass on water

content was similar in all groups. Effects of functional groups were similar as for the four orders (supplementary Table 3.2, 3.3).

Relative water loss referred to water body in our study is not only predicted by *SA/V* ratio, but also to body mass (m), as expected based on the $SA \sim m$ relationship. Insects with the lowest weight in our study were beetles (mean ± sd, 6.7 ± 6.0 mg) and flies (8.7 ± 8.9 mg), whereas hymenopterans (19.1 ± 18.4 mg) and butterflies (18.3 ± 15.5 mg) represented the heavier pollinators (Fig. 3.5c). Smaller, lighter insects lost more water than larger, heavier insects. Different non-linear relationships may describe this pattern. The increase of water loss rate (WL) and body mass (m) appears saturating (supplementary Fig. 3.1), hence $\log(WL) \sim m^b$, with $b < 1$. We focus on $b = 2/3$ in the following analyses, assuming that the surface area is important for predicting water loss, although values below $b = 2/3$ may have even higher explanatory power (supplementary Fig. 3.2). Hence, $m^{2/3}$ significantly predicted water loss at 15°C ($\log(WL) \sim m^{2/3}$, $r^2 = 0.72$, $p < 0.001$, Fig. 3.5a) and also at 30°C ($r^2 = 0.63$, $p < 0.001$). This relationship mirrors the effect of surface area, if all insects had the same shape. To better separate the predictors of relative water loss, i.e. body mass and *SA/V* ratio, we calculated residuals of the regression of *SA/V* ratio over $m^{2/3}$ ($SA/V = -0.24\ m^{2/3} + 4.73$; $r^2 = 0.30$, $p > 0.001$). These residuals (res_1) significantly predicted the unexplained variation in water loss from the above regressions, i.e. the residuals of the $\log(WL) \sim m^{2/3}$ relationship (res_2). Insects with a lower body-mass corrected *SA/V* ratio lost less water than insects with higher *SA/V* (Fig. 3.5b), but res_1 did not differ significantly between orders (Fig. 3.5d). The effect of res_1 was significant at 15°C ($r^2 = 0.20$, $p < 0.001$, $N = 63$ spp.) and at 30°C ($r^2 = 0.15$, $p < 0.001$), confirming that not only body size but also their relative surface area independent of body mass (via res_1) affected water loss of insects. However, the sphericity of the insects, which is constant over their body mass (see Fig. 3.3a), did not explain residual water loss (res_2) ($r^2 < 0.01$, $p = 0.625$).

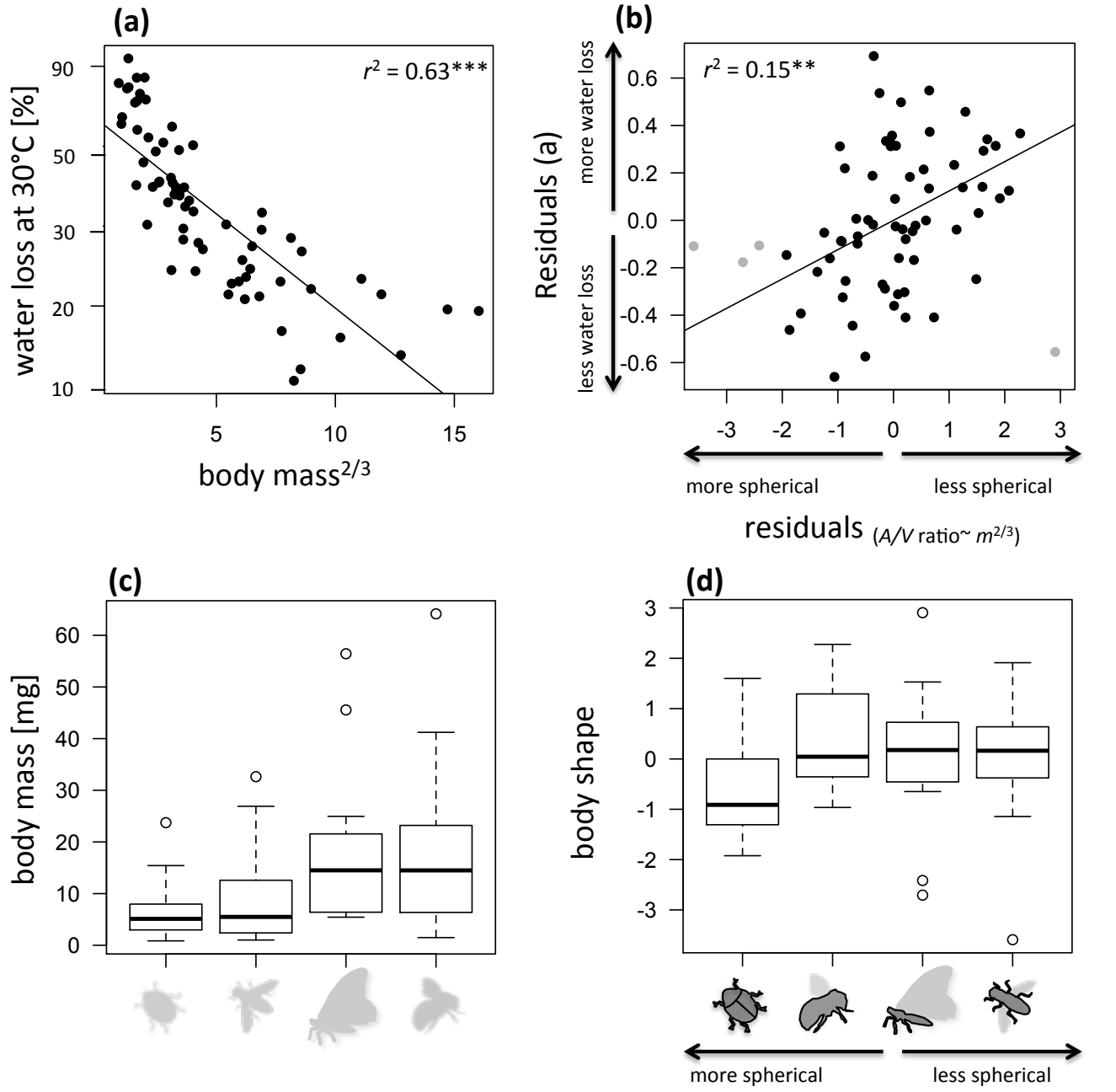

Fig. 3.5: Body mass and body shape as predictors of relative water loss based on water body. **(a)** Effects of body mass ($m^{2/3}$) on water loss at 30°C. We calculated residuals of this linear regression to explain remaining variance in water loss that is not related to body mass, shown as response variable in. **(b)** These residuals were partly explained by variable body shape, given by the residuals of the *SA/V* ratio ~ $m^{2/3}$ regression (more spherical insects have negative residuals). Outliers (grey dots) are considered to be measurement errors and were removed from the analysis, yielding a total of 63 spp. They were defined by the four outlier dots from 4d. Results for the complete set of species (67 spp.) were $r^2 = 0.07$, $p = 0.01$ at 30°C and $r^2 = 0.09$, $p = 0.007$ at 15°C **(c)** Body mass of insect orders. From left to right: beetles, flies, butterflies and hymenopterans. **(d)** Body shape of insect orders (beetles, hymenopterans, butterflies, flies). The dark areas of the order symbols represent actually measured surface area. Wings are excluded because they are considered irrelevant for water loss. Significances: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

The three-dimensional estimates of the surface area may be inaccurate for hairy species with our method. Therefore, we additionally removed particularly hairy taxa (bumblebees, butterflies) and

repeated the analyses. The amount of variance in water loss (res_2) that could be explained by *SA/V* ratio (res_1) was 17 % higher at 30°C: $r^2 = 0.24$) and 21 % higher at 15°C: $r^2 = 0.30$, $N = 49$) than with all individuals (30°C: $r^2 = 0.07$, 15°C: $r^2 = 0.09$).

Discussion

Our results demonstrate that simple predictions based on body mass allometry ($m^{2/3}$) may only inaccurately characterize the relative surface areas of different insect species, since different insects have different shapes. Empirically measured surface areas (*SA*), volumes (*V*) and *SA/V* ratios can be relevant to explain species responses. Here we showed that measured *SA/V* ratios explain variation in water loss of various pollinator species, whereas the affiliation of species to taxonomic orders seems to play a minor role. As expected, insects with a larger *SA/V* ratio lost more water since for a given volume, mass and water content, evaporation via the surface increases. The *SA/V* ratio generally declines with increasing body mass, and the latter can already explain much variance of water loss. The remaining variance in water loss that could not be explained by body mass allometry, was additionally explained by the residuals of the *SA/V* ratio $\sim m^{2/3}$ relationship. More spherical insects with low mass-corrected *SA/V* ratio lost less water than those with higher *SA/V* ratio for a given mass. In contrast, the slope of the $SA \sim V^{2/3}$ relationship alone (*a* in Fig. 3.3 which is volume-independent and can be standardized as sphericity) could not account for residual water loss.

The gravimetric method of measuring water loss is a well-established, easy and cheap method (Hadley 1994) and was used in our study to exemplify the relevance of empirically measured surface areas by 3D-models of different species in comparison to simple body mass allometries. For more exact analyses water losses in an ecophysiological context, newer methods of flow through respirometry (Withers 2001) are often used, allowing precise results of gas exchange characteristic that were beyond the scope of our study.

Inaccuracies of the measured surface of 3D models, which are more pronounced in hairy animals, may have obscured some effects of surface areas or shapes. The structured light method placed the surface partially over the hairs, resulting in a higher surface area. Indeed, water loss of hairy animals showed a weaker relationship with *SA/V* ratios. Surface area may be a greater source of error than volume, as uneven artefacts may increase surface areas erroneously while volume is hardly affected. Our study confirms the allometry of surface area, and their rough relationship to body mass$^{2/3}$ based on measured

values. Body mass$^{2/3}$, has been modeled in other studies of water loss as well, (e.g. Hood & Tschinkel 1990), but no other comparative study has directly quantified surface area or volume so far.

Apart from the *SA/V* ratio, an insect's water balance, particularly its respiratory water loss, may relate to its metabolism. A relationship between water loss and metabolic rate was also hypothesized earlier in a global comparison of insects (Addo-Bediako *et al.* 2001) for xeric species, (Woods & Smith 2010). Metabolic rates per unit body mass generally show a decelerating increase with body mass, consistent with metabolic theory *sensu* (Brown *et al.* 2004). Often, metabolic rates are predicted to be proportional to body mass$^{3/4}$, although values may differ between arthropod taxa (Ehnes *et al.* 2011). This allometric trend may be an alternative or additional explanation of higher water losses in smaller insects. Respiratory water loss is usually neglected in other studies, because cuticular water loss can represent over 80% of the total water loss (Quinlan & Gibbs 2006). However, respiratory water loss increases with temperature and the metabolic rate (Neven 2000). For this reason, respiratory water loss could play an important role in our study, particularly at 30°C. Metabolic rates may change with the specific activity levels of the animals that may occur during the measurements. Both the restrictive bag, as well as the handling during weighing could cause stress, which increases the metabolic rate and thus the respiratory water loss. Using a gravimetric method, we were unable to distinguish cuticular from respiratory water losses, and predictions of different body mass allometries were relatively similar (i.e. different power terms b in the $\log(WL) \sim m^b$ relationship gained similar support, with slight advantages for lower b).

Water loss was also affected by water content of insects in our study. This effect was mainly driven by flies that showed both high water loss and high water content. But flies also had highest *SA/V* ratio, so the potential effect of water content was not independent and relationships were partly confounded.

In our study, water loss of pollinators was highly variable, but not taxon-specific. There were no significant differences in water loss of pollinator orders at both measured temperatures. The smallest animals – and highest water loss rates – in our study were flies (except hoverflies) and beetles, which could be particularly vulnerable to dryness. But species within an order were highly variable in their body mass or *SA/V* as the main factor influencing water loss. Specific differences in water loss may, apart from body mass, *SA/V* allometry and shape, also relate to the thickness and composition of the cuticle that is a more or less effective barrier against water loss (Gibbs 1998). The permeability of the cuticle seems to be mainly driven by the variability of epicuticular hydrocarbons (Gibbs 2002). Hydrocarbon composition varies within species or groups (Raspotnig *et al.* 2008, Jennings *et al.* 2014).

During their exposed diurnal activity and mobility, most pollinating insects need to be particularly well protected against water loss. Unlike some other life-forms, they often do not avoid unfavorable conditions by seeking shelter or favorable microclimates. In contrast, life-forms in microhabitats in which the desiccation potential is greatly reduced (e.g. leaf litter of woods, or in dry regions beneath rocks) may require less protection. For example, millipedes or isopods exhibit higher cuticular transpiration rates and do not possess a well-developed cuticular wax layer (Bursell 1954, Dwarakanath & Job 1965), but see (Crawford 1972). In addition to their more sheltered habitats, they often restricting their activity to nighttime when temperature and humidity are more favorable. In contrast, cuticular transpiration rates of tenebrionid beetles are particularly low. Their low permeability is in part due to an abundance of epicuticular surface waxes but also due to the presence of an air–filled space known as subelytral cavity that separates the abdominal tissues from the surrounding air and thus provides an effective boundary layer (Ahearn & Hadley 1969). Also active water retention by cuticular and spiracular regulation are causes of low water loss (Ahearn & Hadley 1969).

It is generally observed that animals living in xeric habitats are better adapted to low water availability than animals from mesic habitats. The mesic Argentine ant showed significantly higher rates of water loss and cuticular permeability compared to four ant species native to dry environments. Physiological limitations may therefore be responsible for restricting the distribution of this species in seasonally dry environments (Schilman *et al.* 2005). Also bees in xeric habitats, particularly small species can be water stressed when foraging. Hence, they avoid heat and are more likely adapted to cold dawns and dusks (Willmer & Stone 1997).

Temperature and water availability, alone or in combination, have a strong impact on the geographical distribution, abundance and behavior of insects (Addo-Bediako *et al.* 2000). The climatic characteristics of their environment, particularly temperature, can determine the extent of insect activity, as shown for pollinators elsewhere (Willmer & Stone 2004). With respect to projections of effects of global warming, behavioral responses of pollinators to avoid extreme temperatures have the potential to significantly reduce pollination services (Corbet *et al.* 1993).

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Supplementary Information

Supplementary Table 3.1: Measured surface area and volume and calculated sphericity of 67 pollinator species of four orders.

order	species	surface area	volume	sphericity
Coleoptera	*Cassida spec.*	21.65	4.95	1.54
	Chrysolina fastuosa	64.31	11.07	2.68
	Coccinella septumpunctata	149.15	79.80	1.66
	Cryptocephalus moraei	41.34	9.43	1.92
	Cryptocephalus sericeus	185.18	118.92	1.58
	Cryptocephalus vittatus	32.18	14.33	1.13
	Cuculionidae spec.	28.71	9.68	1.31
	Labidostomis longimana	71.33	27.91	1.60

order	species	surface area	volume	sphericity
Coleoptera	*Oedemera femorata*	149.24	43.98	2.48
	Oedemera nobilis	104.68	23.89	2.61
	Oedemera virescens	41.84	7.31	2.30
	Oxythyrea funesta	198.36	117.92	1.71
	Pseudovadonia livida	69.65	22.72	1.80
	Rhagonycha fulva	120.55	41.93	2.07
	Stenurella melanura	187.69	59.71	2.54
Diptera	*Botanophila fugax*	65.21	10.67	2.78
	Cheilosia gigantea	97.24	28.00	2.18
	Chloromyia formosa	63.03	15.13	2.13
	Chrysogaster virescens	61.65	20.51	1.70
	Chrysotoxum latilimbatum	161.43	76.15	1.86
	Dioctria atricapilla	99.32	24.23	2.45
	Dolichopus ungulatus	58.80	13.24	2.17
	Empis livida	280.43	88.11	2.93
	Episyrphus balteatus	12.69	51.65	0.19
	Eristalis tenax	295.43	122.39	2.48
	Helina confinis	47.06	9.48	2.17
	Helophilus trivitatus	239.10	104.51	2.23
	Lucilia sericata	82.54	27.53	1.87
	Minettia fasciata	28.33	4.43	2.17
	Musca domestica	187.85	43.11	3.16
	Orellia falcata	69.29	16.04	2.25
	Pollenia similis	51.11	11.95	2.02
	Sapromyza quadripunctata	21.11	4.83	1.53
	Sarcophaga carnaria	294.61	125.27	2.43
	Sarcophaga haemorrhoa	483.80	148.61	3.57
	Sarcophaga lehmanni	226.82	74.13	2.66
	Scaeva pyrastri	267.14	81.47	2.94
	Soleria pacifica	87.31	14.63	3.02
	Solieria vacua	63.21	11.71	2.53
	Sphaerophoria interrupta group	63.03	12.74	2.39
Hymenoptera	*Ambyletes armatorius*	361.15	70.18	4.39
	Anthidium byssinum	191.49	100.83	1.83
	Arge cyanocrocea	54.24	9.65	2.48
	Athalia rosae	33.41	8.02	1.72
	Bombus lapidarius	182.32	166.40	1.25
	Bombus sylvarum	565.78	156.97	4.02
	Bombus terrestris	432.94	834.77	1.01
	Coelioxys cf elongata	183.65	57.34	2.55
	Ichneumonidae spec	68.06	10.45	2.95
	Lasioglossum leucozonium	122.86	27.64	2.78
	Nomada succinata	188.90	55.91	2.67
	Sphecidae spec.	128.46	33.75	2.54
	Tenthredo notha	154.17	57.75	2.13
Lepidoptera	*Aglais urticae*	480.39	150.45	3.51
	Araschnia levana	178.37	52.72	2.62
	Argynnis aglaja	411.30	224.06	2.31
	Coenonympha pamphilius	103.43	24.95	2.50
	Coenonympha spec.	95.52	21.89	2.52
	Maniola jurtina	65.43	141.33	0.50
	Melanargia galathea	331.72	92.26	3.36
	Melitae britomartis	99.21	28.26	2.21
	Mompha spec.	72.72	22.53	1.89
	Pieris rapae	63.24	132.15	0.50
	Plebeius idas	116.36	38.80	2.10
	Polyommatus icarus	98.20	28.31	2.19

order	species	surface area	volume	sphericity
	Thymelicus sylvestris	187.06	40.30	3.29
	Zygena filipendulae	342.76	64.41	4.41

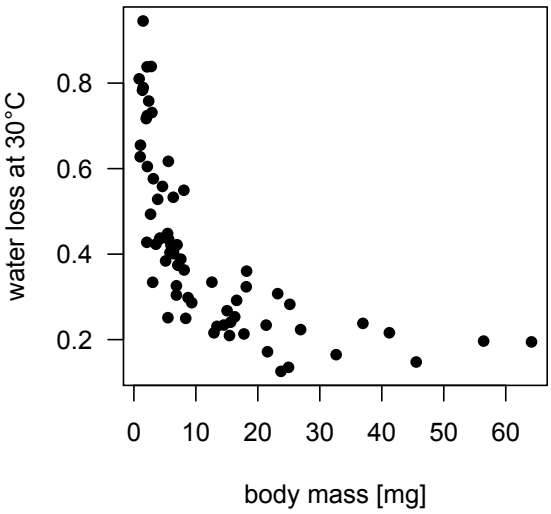
Supplementary Table 3.2: Effects of body mass, surface area to volume ratio and insect taxonomic groups (see below) on water content and water loss (at 15°C and 30°) of pollinators. Predictors with $p < 0.05$ were considered to be significant and bold print. To analyze reasons of different water loss at a lower taxonomic level, we subdivided two orders into so-called "taxonomic groups". We divided Diptera ("flies") into „hoverflies" (Syrphidae) and „other flies" because of their different shape and acknowledging that hoverflies are a focal pollinator group in many studies. Whereas hoverflies have a flattened shape, other flies are more spherically. Moreover within the order Hymenoptera „bumblebees" were separated from „other hymenopterans" because of the different body size, shape and their pronounced hairiness. Other hymenopterans comprised other wild bees, wasps and Symphyta.

	df_{num}	df_{den}	*F*	*P*
water content [%]				
body mass	1	48	34.2	**< 0.001**
taxonomic group	5	48	4.7	**0.002**
mass × taxonomic group	5	48	1.0	0.439
water loss at 15°C [%]				
SA/V ratio	1	48	61.1	**< 0.001**
taxonomic group	5	48	1.3	0.397
SA/V ratio× taxonomic group	5	48	3.4	**0.010**
water loss at 30°C [%]				
SA/V ratio	1	48	57.8	**< 0.001**
taxonomic group	5	48	1.5	0.217
SA/V ratio× taxonomic group	5	48	2.6	**0.034**

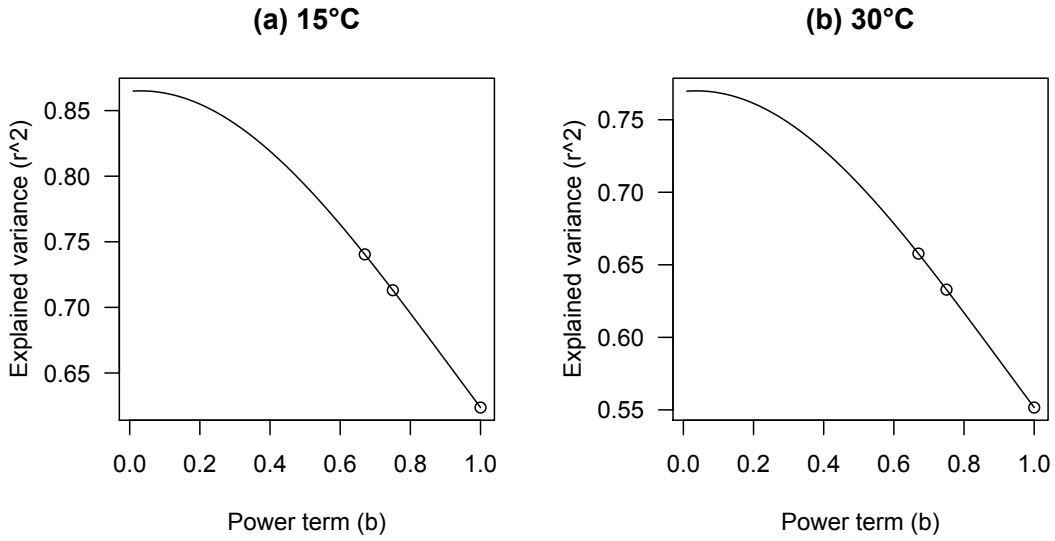
Supplementary Table 3.3: Differences in water content, water loss (15°C and 30°C) and surface area to volume ratio of insect taxonomic groups within orders. Results are from two sample welch t-tests. Predictors with $p < 0.05$ were considered to be significant and bold print.

	t	*df*	*P*
water content [%]			
hoverflies - other flies	1.2	22.8	0.253
bumblebees - other hymenopterans	1.9	10.9	0.083
water loss at 15°C [%]			
hoverflies - other flies	1.9	15.5	0.064
bumblebees - other hymenopterans	-2.7	9.0	**0.026**
water loss at 30°C [%]			
hoverflies - other flies	2.1	14.0	0.058
bumblebees - other hymenopterans	-3.3	9.4	**0.009**
SA/V ratio			
hoverflies - other flies	2.9	11.8	0.014
bumblebees - other hymenopterans	-2.2	2.9	0.124

Supplementary Fig 3.1: Relationship between water loss and body mass of pollinators.



Supplementary Fig. 3.2: Explanatory power (r^2) of different power terms (b) of body mass (m) to predict water loss rate (WL) at 15°C **(a)** and at 30°C **(b)**. The underlying linear model is log(WL) $\sim m^b$. The three points highlight b = 2/3 (square-cube law), b = 3/4 (metabolic theory) and b = 1 (linear relationship).



CHAPTER IV

Land-use intensity as a filter of morphological trait composition: pollinator communities in meadows and pastures

Sara Kühsel, Nico Blüthgen

Abstract

Insect communities are known to be vulnerable to land-use intensification, and they respond with diversity declines and compositional changes. How land-use as an environmental filter leads to changes in the composition of functional traits of species has been rarely investigated. We recorded pollinator communities on 40 grassland sites along a land-use gradient from extensively used to high fertilized and frequently mown and/or grazed grasslands. Pollinator diversity was high and was largely unaffected by land-use intensity. We measured morphological characteristics of 476 pollinator species. Community-weighted means of body size, hairiness, relative wing size, and relative proboscis length decreased with land-use intensity, whereas relative leg length increased. Relative eye size, antenna length, mandible length and femur length were unaffected of land-use. Trait heterogeneity, i.e. the variability of traits was not reduced by increasing land-use intensity; instead, variation in size of head and eyes increased, whereas relative wing size decreased. Traits differed between insect orders. Hence, shifts in trait means strongly correspond to shifts in relative abundance of insect orders, whereas consistent land-use changes were rarely found within an order. These findings highlight that functional traits may be more sensible indicators of land-use intensity than species diversity alone, and many of these traits can be relevant for ecosystem functionality.

KEYWORDS: community composition, community weighted mean, environmental filtering, land-use change, morphological traits

Introduction

It is increasingly recognized that species functional traits rather than species richness alone are meaningful predictors of ecosystem functioning (Hooper *et al.* 2005; Petchey & Gaston 2006). Hence, there is a growing interest in the diversity of species traits as a tool to study the role of organisms in ecosystems, responses to environmental changes and potential impacts of community changes and losses of species (Petchey & Gaston 2006; Flynn *et al.* 2009). For example, the richness of functional groups can be a better predictor of biomass accumulation in grasslands (Tilman *et al.* 1997), diversity loss due to nitrogen fertilization (Suding *et al.* 2005) and decomposition rates (Scherer-Lorenzen 2008) than species richness alone. Whereas conservation management often focuses on rare species to investigate changes in ecosystems, these rare species alone are no powerful indicators of the dynamics of entire communities and ecological consequences (Lindenmayer & Likens 2010).

It has long been observed that species communities differ in response to the conditions of the habitat they occupy. The underlying reasons for this pattern are still a key issue in ecology (McGill *et al.* 2006; Argawal *et al.* 2007). It has been hypothesized that environmental conditions may act as a filter for species composition: species have competitive advantages in specific habitats if their functional traits (morphological, physiological or life-history traits) are compatible with the environmental conditions that they face in their habitat (Southwood 1988; Keddy 1992). Filtered traits may also cause further changes: when species with particular traits are lost, this may translate into changes in ecosystem functioning and services (Larsen, Williams & Kremen 2005; Cardinale *et al.* 2012). Consequently, quantifying the links between specific environmental conditions and functional traits of its species can enhance our understanding of ecosystem functioning and community composition (Lavorel & Garnier 2002; McGill *et al.* 2006; Green, Bohannan & Withaker 2008; Lebrija-Trejos *et al.* 2010; Menezes, Baird & Soares 2010; Webb *et al.* 2010).

The assumption that environmental filtering appears is supported by studies of several species traits. Bee species responses to environmental disturbance can be predicted by ecological and life-history traits (Williams *et al.* 2010). For butterflies it is known that species life-history traits predict phenological response to climate change (Diamond *et al.* 2011) and that traits also can be used to explain recent shifts in butterfly distribution with ongoing climate change (Pöyry *et al.* 2009). And despite their large mobility, life-history traits of syrphid fly communities respond to changes in environmental variables at small scales (Dziock 2006). In addition to life-history traits also further traits can be affected by environmental variation. Ant communities showed shifts in physiological traits (thermal tolerance, desiccation resistance) and morphological traits (head size, leg length) in response to temperature and climate change (Wiescher, Pearce-Duvet & Feener 2012). Body sizes of ground beetles tend to be larger in highly disturbed habitats (Ribera *et al.* 2001; Alaruikka *et al.* 2002; Magura, Tóthmérész & Lövei 2006), showing that not only environmental conditions, but also habitat characteristics can affect species traits. Increasing land-use intensity is suggested to cause non-random pollinator species losses, with species with traits like narrow diet breadth, large body size, solitary behavior and non floral larval food resources get lost first (Rader *et al.* 2014). From studies on grassland plants it has been confirmed that community traits change with land-use intensity and can thus act as additional indicator for land use impacts (Garnier *et al.* 2007; Pfestorf *et al.* 2013).

However, our knowledge about how a wide spectrum of community traits rather than single species traits change with land-use, is still fragmentary. Trait measurements and compilations are time-consuming, and useful databases only exist for limited taxa. Identifying common functional traits that affect the vulnerability of species to habitat changes may improve our insight into community

responses beyond simple changes in species composition and diversity.

Communities of pollinating insects provide an ideal study system for examining trait–habitat relationships, as they are known to differentially respond to land-use changes (Kremen *et al.* 2007; Potts *et al.* 2010), and since they cover a broad taxonomic spectrum of species particularly from the four most species-rich orders of holometabolous insects (dipterans, hymenopterans, butterflies and beetles). Species from all four orders play a key role as pollinators in various ecosystems and contribute both to pollination of wild plants (Ashman *et al.* 2004; Aguilar *et al.* 2006) as well as crops (Klein *et al.* 2007; Ricketts *et al.* 2008).

The intensification of land use leads to a shift from complex natural ecosystems to simplified agricultural systems and is considered as a major cause of global biodiversity loss (Matson *et al.* 1997; Tscharntke *et al.* 2005). Intensive land-use in grasslands is defined as high fertilizer application, frequent mowing or grazing and in certain cases pesticide entry (Blüthgen *et al.* 2012). Grazing and cutting at least temporarily remove floral food resources and affect pollinators accordingly (Kruess & Tscharntke 2002). They may also lead to impoverished grasslands with structurally homogenous food resources for pollinators (Weiner *et al.* 2011).

Identifying how pollinator traits are linked with land-use managements is a necessary step to assess how ongoing land-use intensification may alter pollinator communities and ecosystem processes. Thus, this study addresses the investigation of the relationship between land use management, species diversity and abundance, and species' functional traits in managed grassland ecosystems in two different regions in Germany.

We concentrate on body size and relative morphological traits, as they are the basis for functional performance of species und can affect metabolism (Brown *et al.* 2004), sensitivity to climatic changes (Addo-Bediako, Chown & Gaston 2001), reproductive rate (Williams *et al.* 2010), mobility (Thomas 2000; Gabriel *et al.* 2010), or diet selection (Müller 1995). We used community-weighted mean values for each trait, allowing us to consider species abundances that are important for the assessment of trait composition (de Bello *et al.* 2007). In addition to the mean, the variability of traits in a community is important to define the community niche breadth reflected by the trait, with important consequences for functional resilience (McGill *et al.* 2006; Kühsel & Blüthgen 2015). We used the coefficient of variation (calculated with mean and standard deviation of traits) as measure for variability. It is also an indicator of functional diversity, which as well represents the distribution of species and their abundance in a functional space, when all species of a multi-trait space are considered (Villéger,

Mason & Moulliot 2008).

The objectives of this study were to: (1) identify changes in functional traits of pollinator communities in response to varying land-use intensity (2) to investigate whether some communities have narrower trait variability than others and (3) to find out whether traits correspond to taxonomical groups (the four insect orders). To answer these questions we measured morphological traits of over 476 pollinator species that were recorded from 40 grassland plots along a land-use gradient.

Material and Methods

Study sites

We collected data between May and September 2012 in two regions: the Hainich-Dün region in central Germany (10°10'24"–10°46'45" E / 50°56'14"–51°22'43" N) and the Schwäbische Alb in southwestern Germany (09°10'49"–09°35'54" E / 48°20'28"–48°32'02" N). The plots were part of the Biodiversity Exploratories project (Fischer *et al.* 2010). We selected 40 plots along a land-use gradient from semi-natural to intensively managed grasslands. Land use can be characterized for each plot by a compound land-use intensity index (LUI, (Blüthgen *et al.* 2012) that integrates intensity of fertilization, mowing frequency and grazing intensity. For our analyses we use an average LUI of 2011 and 2012 to also consider land-use management of the previous year, which may have a direct influence on the current pollinator community as they mostly have an annual life cycle. Mowing frequency across both years ranged from 1 – 3 cuts on meadows and mown pastures (12 unmown pastures in both years), grazing intensity (GI = [live stock units × days of grazing per year]/ha) from 15.8 – 757.1 on pastures and mown pastures (5 ungrazed meadows), and fertilization intensity, characterized by the amount of applied fertilizer, from 3 - 245.2 kg N /ha (21 unfertilized plots). The three land-use components were standardized globally across regions to calculate the LUI. Land-use factors were not independent of each other. Whereas grazed pastures were rarely mown or fertilized (Spearman rank tests, grazing *vs.* mowing intensity: $r = -0.527$, $p < 0.0001$, grazing *vs.* fertilization: $r = -0.401$, $p = 0.010$), mowing and fertilization often occurred together ($r = 0.721$, $p < 0.0001$).

Pollinator sampling

We recorded pollinators on 16 plots in the Schwäbische Alb and 24 plots in the Hainich. Size of grassland plots varied between 187.1 and 1.4 ha (mean ± sd, 28.4 ± 47.7). The plots were observed repeatedly between one (four cases) and 13 times (median: 4 observation days per plot, corresponding to a total observation time mean of 24 h per plot). Each time a transect of about 300 m^2 per plot was observed for six hours between 08.00 and 14.00 (methods comparable with Weiner *et al.* 2014). The transect was divided into eight sectors of 25 m length and three meter width. Each sector was observed for 15 min, three times a day. During these transects walks all flower visitors that touched reproductive parts of the flowers, and thus are potential pollinators, were collected. All animals were identified to species level either directly at the site or later by taxonomists. All flower visitors recorded in this study are known to pollinate flowers in general (but not necessarily all herbs in these grasslands). Non-pollinating taxa (e.g. bugs) were excluded; Thysanoptera and pollen beetles (*Meligethes sp*.) were not counted, because they can occur in large quantities, often hidden within flowers, and could therefore not be reliably quantified across different plants.

Morphological traits

We measured morphological traits for 476 pollinator species (60 males, 131 unsexed individuals, 285 females). Most species were Diptera (327 spp.), followed by Hymenoptera (81 spp.), Coleoptera (46 spp.) and Lepidoptera (22 spp.). Of the total number of pollinator species observed in the field, 35 rare species (representing 126 or 0.8% of 14873 individuals) could not be measured and were not considered in the analysis. Males were only measured if females were unavailable (see supplementary Table 4.1). We measured 16 morphological traits (provided in Fig. 4.1) of a single specimen per species and additionally estimated hairiness. We took pictures at different magnification and from various perspectives to measure species traits with the stereomicroscope Motic® SMZ – 168 and an associated digital camera and software. When it was not possible to measure a trait, for example due to broken antennas or missing legs of a species for which no other specimen were available, the gap was filled with the mean genus value (14 cases), or (if it was the only individual of a whole genus) with the family mean (5 cases).

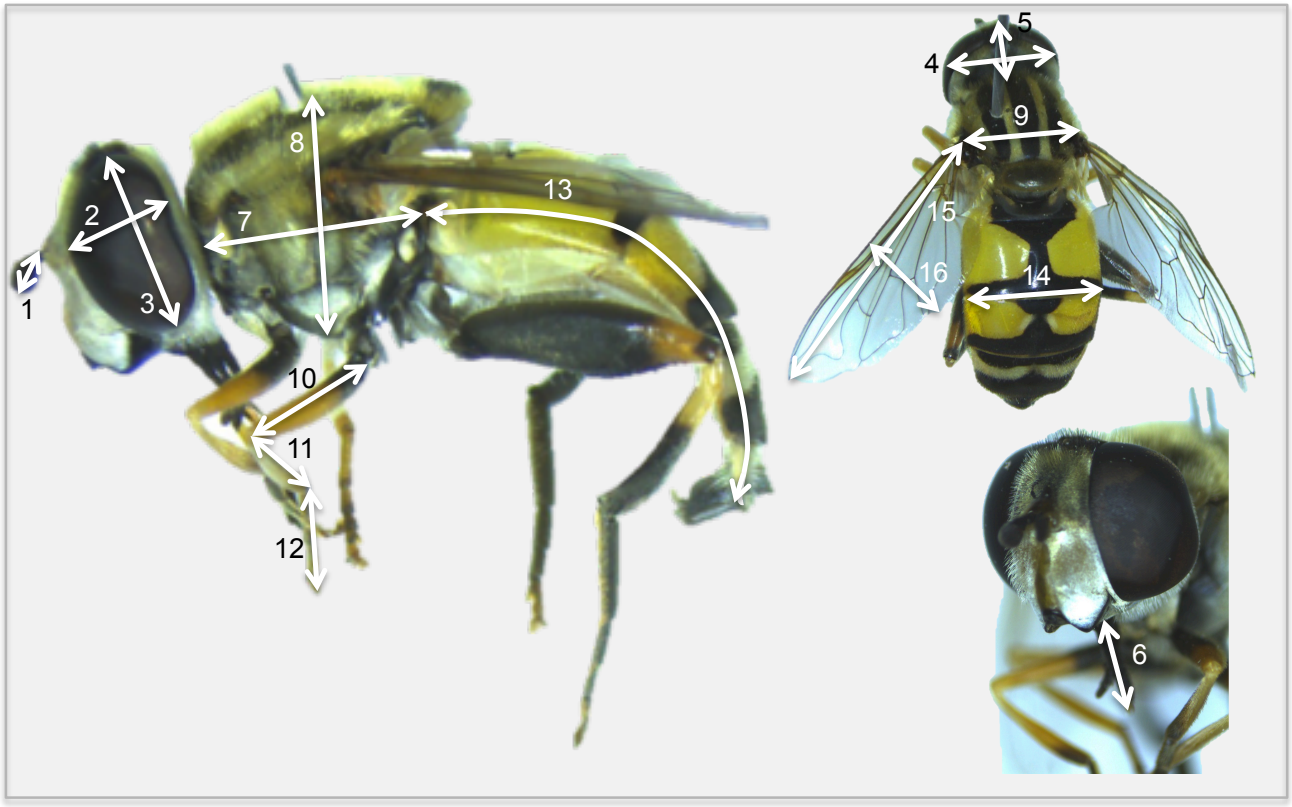


Fig. 4.1: Overview of measured morphological traits. (1) antenna length, (2) eye width, (3) eye length, (4) head width, (5) head length, (6) proboscis length, (7) thorax length, (8) thorax height, (9) thorax width, (10) femur length, (11) tibia length, (12) tarsi length, (13) abdomen length, (14) abdomen width, (15) forewing length, (16) forewing width. When hindwings and mandibles were present they were also measured (length and width). The numbering starts at the head and continue towards the abdomen.

Table 4.1: Mean and standard deviation (sd) of morphological traits of 476 pollinator species.

Trait	Definition [1])	Coleoptera	Diptera	Hymen-optera	Lepid-optera	**All**
thorax volume	$T_{vol} = 4/3\ \pi \cdot l_T/2 \cdot w_T/2 \cdot h_T/2$	2.47	2.44	3.10	3.82	2.62
						(1.17)
rel. head size	$(4/3\ \pi \cdot l_H/2 \cdot w_H) / T_{vol}$	1.39	1.77	2.13	2.25	1.81
		(0.60)	(0.74)	(0.82)	(0.63)	(0.76)
rel. antenna length	$l_{antenna} / H_{geo}$	2.26	0.45	1.85	4.71	1.07
		(0.91)	(0.31)	(0.45)	(1.21)	(1.17)
rel. eye size	$(2 \cdot l_{eye} \cdot w_{eye})/(l_H \cdot w_H)$	0.30	0.91	0.52	0.74	0.77
		(0.16)	(0.28)	(0.16)	(0.22)	(0.33)
rel. proboscis length	$l_{proboscis} / H_{geo}$	1.21	4.27	9.50	48.80	6.96
		(7.01)	(3.64)	(11.81)	(40.25)	(14.67)
rel. mandible length	$l_{mandible} / H_{geo}$	0.37	NA	0.58	NA	0.47
		(0.13)	(NA)	(0.08)	(NA)	(0.12)
rel. wing size	$(l_{w1} \cdot w_{w1} + l_{w2} \cdot w_{w2}) / T_{vol}$	2.37	2.77	2.32	20.90	3.51
		(1.59)	(2.19)	(1.51)	(12.37)	(5.08)
rel. leg length	$(l_{femur} + l_{tibia} + l_{tarsi}) / T_{geo}$	1.81	2.53	2.00	2.72	2.38
		(0.47)	(0.92)	(0.44)	(0.40)	(0.85)
rel. femur length	l_{femur} / T_{geo}	0.35	0.34	0.29	0.33	0.33
		(0.04)	(0.02)	(0.02)	(0.02)	(0.03)
hairiness	mean %, see Methods	25.61	14.83	55.88	93.96	26.62
		(15.85)	(13.68)	(21.14)	(2.32)	(26.14)

[1]) l = length, w = width, h = height, H = head, T = thorax, A = abdomen, $w1$ = forewing, $w2$ = hindwing; hence e.g. l_T = thorax length. Length references: $H_{geo} = (l_H \cdot w_H)^{1/2}$, $T_{geo} = (l_T \cdot w_T \cdot h_T)^{1/3}$, NA = not applicable.

We selected the volume of the thorax as a standardized measure of body size. Thorax volume (T_{vol}) was estimated from length (l_T), width (w_T) and height (h_T) (Fig. 4.1), assuming that it is an ellipsoid: $T_{vol} = 4/3\ \pi \cdot l_T/2 \cdot w_T/2 \cdot h_T/2$. We focused on the thorax rather than on total body length commonly reported in the literature, since the latter depends on the angle between the three tagmata, and the abdomen is often compressed or overexpanded, rendering body length an inaccurate size measure. Since some insects were pinned and others stored in alcohol, we could not reliably determine body mass.

To describe changes in traits independent of trends in total body size, we focused on relative trait ratios rather than absolute size measures (Table 4.1). For this purpose, we defined two reference lengths for the appendages on the two tagmata head and thorax: the geometric mean of the head length, based on its length l_H and width w_H as $H_{geo} = (l_H \cdot w_H)^{1/2}$, and the geometric mean of the thorax

as $T_{geo} = (l_T \cdot w_T \cdot h_T)^{1/3}$. This enabled us to compare lengths with lengths instead of comparing length with volume, which would create a nonlinear bias with increasing size. We thus standardized the length of antenna, mandibles and proboscis against mean head length (H_{geo}). Mandible width was strongly correlated with mandible length (Spearman rank, $r = 0.994$, $p < 0.0001$) and not considered as an additional trait. Relative eye size was defined based on the product of eye length l_E and width w_E as $E_{area} = 2 \cdot l_E \cdot w_E$ and standardized against the head area $H_{area} = l_H \cdot w_H$. Leg length was standardized against the thorax length (T_{geo}). Within a leg, femur length was related to tibia plus tarsae. Wing size was defined as the ratio of wing area of fore- and hindwings versus thorax volume; elytra of beetles were not considered, as these are only passively involved in flying. Relative head size was estimated as volume ratio between head and thorax based on ellipsoids. All equations are provided in Table 4.1.

In addition to linear or geometric measures above, we estimated hairiness of pollinators. To achieve a representative value for the exposed surface of the insect, the hairiness of the front side of the head (H_w), the upper thorax (T_{U_w}), the lateral thorax (T_{L_w}), the upper abdomen (A_{U_w}) and the lower abdomen (A_{L_w}) were estimated in percent. From these values for each body part, the weighted mean hairiness was obtained, weighted by the estimated surface area of each body part. To represent two areas per tagma, the respective weights are as follows: $H_w = 2 \cdot l_H \cdot w_H$, $T_{U\_w} = l_T \cdot w_T$, $T_{L\_w} = l_T \cdot h_T$, $A_{U\_w} = A_{L\_w} = l_A \cdot w_A$.

Based on the measurements of morphological traits we calculated body size (thorax volume), hairiness and 8 further traits (relative to body size) that were used in the analyses: head size, proboscis length, mandible length, antenna length, eye size, leg length, femur length and wing area.

Statistics

Statistics were conducted in R 2.15.1 (R Development Core Team 2012). We calculated a community weighted mean and CV (coefficient of variation) for each trait, based on all species found in this year on a specific plot, weighted by the proportional abundance of each species in the community p_S. The community weighted mean trait μ_C of the set of species is defined as

$$\mu_C = \sum_{S=1}^{\max(S)} p_S \cdot t_s$$

were t_s is the specific trait values for each species. The weighted coefficient of variation (CV_C) of the community in t_s is defined as

$$CV_C = \frac{\sigma_\mu}{\mu_C}, \text{ with } , \sigma_\mu = \sum_{s=1}^{\max(s)} p_s \cdot (t_S - \mu_C)$$

Community mean traits and variation of traits (*CV*) were unaffected of pollinator diversity on the plots (see supplementary Table 4.2). To assess the intraspecific variation in traits, particularly in relation to the interspecific variation, we additionally measured 5 individuals of a fly (*Syrphus ribesii* L.) and a bee (*Andrena haemorrhoa* Fabr.). We then compared the coefficient of variation of functional traits of both repeatedly measured species with the variation in traits across all species. Mean CV of traits within the species was about one-third of the CV level across species (34.9% in *A. haemorrhoa*, 27.2% in *S. ribesii*, respectively; see supplementary Table 4.3).

We additionally calculated residuals of the linear regressions of community mean traits over the proportion of flies per plot and tested the fixed and interaction effects of land-use intensity and region on residuals instead of μ_C or CV_C. By including the residuals of functional traits, we de-trended the land-use effects from the most prominent change in taxonomic composition, i.e. an increasing proportion of flies. However, the usage of residuals had no effect on the general conclusions (see supplementary Table 4.4). We thus used the more simple linear models to test how μ_C or CV_C were predicted by region and land-use intensity. To better separate effects of land-use intensity, we additionally analyzed effects of mowing frequency, grazing intensity and fertilization intensity on community mean traits and CV separately with linear models in addition to the compound LUI index.

Differences in trait means of species within orders were analyzed by ANOVAs and following Tukey post-hoc tests. With linear models we assessed the effects of land-use intensity on pollinator abundance and diversity. As a measure of effective diversity we used $e^{H'}$ for the analysis.

To identify drivers of land-use effects we analyzed species traits with principal component analysis (PCA). Hairiness was excluded from the PCA, because it is difficult to compare with other traits of body lengths. Sizes of thorax and head were also not included in PCA, as they serve as reference for the calculation of other morphological traits. Hence, seven traits were combined in the PCA: antenna length, proboscis length, eye size, mandible length, wing size, leg length and femur length. All data were log-transformed, and proboscis length was double log-transformed to achieve normality. We used the PCA1 value of every species to calculate a weighted PCA1 mean for each community.

Results

General findings

Pollinator diversity did not change with land-use intensity in both regions (LUI: $F_{1,36} = 0.1$, $p = 0.81$, region: $F_{1,36} = 3.8$, $p = 0.056$). Also pollinator abundance did not change (LUI: $F_{1,36} < 0.1$, $p = 0.914$, region: $F_{1,36} = 3.2$, $p = 0.081$). Instead, the composition of the pollinator community changed considerably. The proportion of flies increased significantly with land-use intensity ($F_{1,38} = 5.1$, $p = 0.029$) except hoverflies that showed an opposite trend ($F_{1,37} = 3.3$, $p = 0.079$). In turn, the proportion of butterflies decreased with land-use intensity ($F_{1,26} = 9.9$, $p = 0.004$), as well as the bees in the Schwäbische Alb ($F_{1,14} = 13.3$, $p = 0.003$), but not in the Hainich ($F_{1,22} = 0.2$, $p = 0.698$). There was no consistent change in the proportion of other hymenopterans and beetles.

Thorax volume as a measure of body size decreased with land-use intensity; pollinators at the upper end of the land-use gradient had only half of the size of pollinators at the least intensive plots (Fig. 4.2). The decreasing body size was driven by the negative effects of mowing frequency and fertilization intensity; there was no significant effect of grazing intensity on thorax volume.

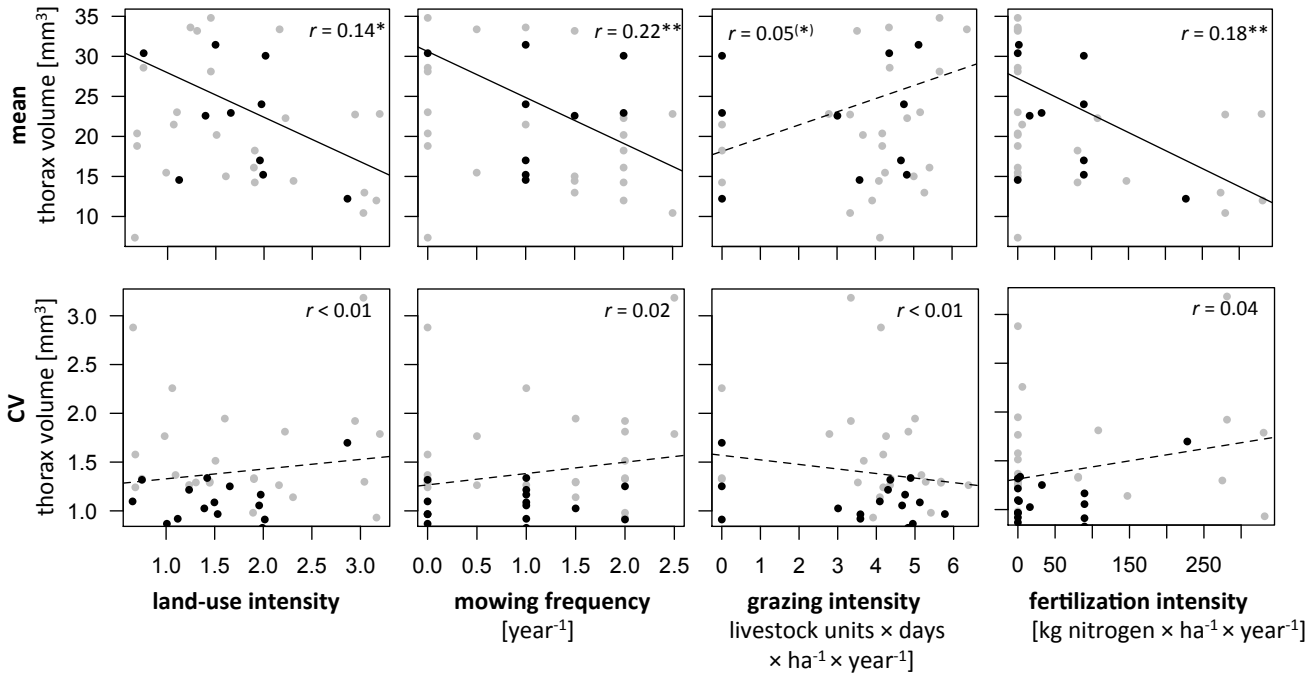


Fig. 4.2: Linear models of community weighted means and coefficients of variation (CV) of body size (thorax volume) and land-use intensity (averaged for 2011 and 2012). In addition to the compound land-use intensity (LUI) index, mowing frequency, log grazing intensity and fertilization intensity were analyzed separately. Different regions are indicated by different colors (Hainich: grey, Schwäbische Alb). Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships. Significances: $^{**}p < 0.01$, $^{*}p < 0.05$, $^{(*)}p < 0.01$.

To get a general overview over relative morphological traits of pollinator species we conducted a principal component analysis. Its first axis (PCA1) explained 38.6 % of the variation of all traits included (PCA2: 21%, PCA3: 14%). As expected, species were arranged in groups that were defined by the four insect orders (Fig. 4.3). There was a significant change of the first principal component (PCA1) with land-use intensity, but not with PCA2 (Table 4.2, Fig. 4.4). Mowing frequency but not grazing and fertilization intensity significantly affected PCA1. We correlated all morphological traits to the combined response variable (principal component, PCA1) with Pearson's correlations. Relative wing area, antenna length and proboscis length were significantly related to PCA1 but not femur and leg length, eye size and mandible length (see supplementary Fig. 4.1).

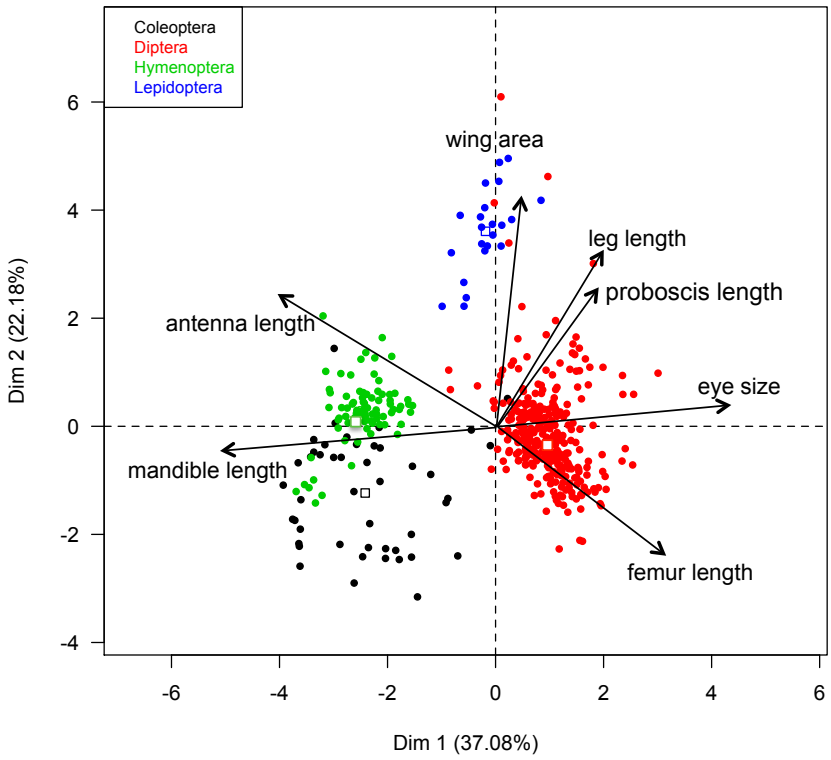


Fig. 4.3: Principal component analysis of pollinator species traits.

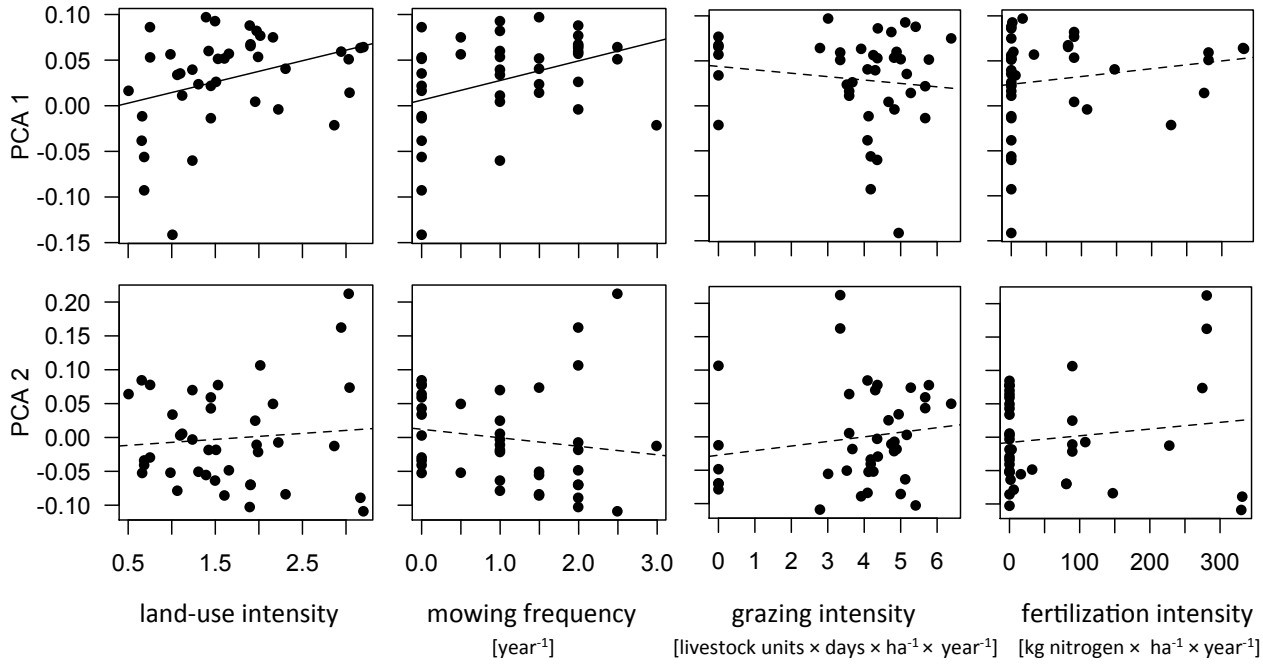


Fig. 4.4: Linear models of community weighted means of PCA axis 1 and 2 and land-use intensity (averaged for 2011 and 2012). In addition to the compound land-use intensity (LUI) index, mowing frequency, log grazing intensity and fertilization intensity were analyzed separately. Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships.

Community mean and variation of functional traits

We investigated fixed and interaction effects of land-use intensity and region on mean and variation of community traits. Among the selected traits, land-use intensity had a significant negative effect on relative size of head (18% reduced, Fig. 4.5), wing area (57%), hairiness (45%), proboscis length (64%) and leg length (11%) and tended to reduce antenna length and eye size (Table 4.2). Land-use effects on hairiness and leg length were found only in the Schwäbische Alb but not in the Hainich.

Table 4.2: Fixed and interaction effects of land-use intensity index (LUI) and Exploratory (region) on community weighted mean of relative traits and coefficient of variation (CV) of relative traits (40 communities). Results are from linear models. Significant values are highlighted in bold.

community mean trait	LUI		region		LUI × region	
	F	*p*	*F*	*p*	*F*	*p*
PCA 1	4.7	**0.037**	0.3	0.581	< 0.1	0.860
PCA 2	0.4	0.553	2.9	0.099	3.4	0.075
body size (thorax volume) [1)]	10.8	**0.002**	7.8	**0.008**	1.8	0.183
rel. head size	7.4	**0.01**	2.8	0.105	2.8	0.100
rel. antenna length	3.4	0.076	2.2	0.144	3.9	0.055
rel. eye size [1)]	2.9	0.095	5.5	**0.024**	0.2	0.621
rel. proboscis length	12.3	**0.001**	3.5	0.069	2.5	0.123
rel. mandible length	0.4	0.507	2.9	0.097	6.5	**0.016**
rel. wing size	6.1	**0.018**	1.0	0.325	< 0.1	0.998
rel. leg length[2)]	5.4	**0.025**	2.3	0.136	18.5	**< 0.001**
rel. femur length	0.9	0.355	7.0	**0.012**	0.4	0.544
hairiness	7.6	**0.009**	1.8	0.184	9.9	**0.003**

community CV trait	LUI		region		LUI × region	
	F	*p*	*F*	*p*	*F*	*p*
body size (thorax volume) [1)]	2.5	0.122	2.5	0.122	1.7	0.195
rel. head size	9.6	**0.004**	0.1	0.788	2.0	0.171
rel. antenna length	0.1	0.742	5.3	**0.027**	2.4	0.131
rel. eye size [1)]	5.7	**0.023**	3.0	0.091	0.3	0.600
rel. proboscis length	0.5	0.479	2.0	0.170	0.1	0.773
rel. mandible length	1.3	0.253	2.9	0.098	0.2	0.652
rel. wing size	5.5	**0.025**	0.1	0.729	0.4	0.554
rel. leg size	1.8	0.193	3.1	0.088	3.9	0.057
rel. femur length [3)]	1.7	0.198	5.8	**0.022**	0.7	0.402
hairiness	4.4	**0.043**	1.7	0.205	15.2	< **0.001**

[1)] Size of thorax and eye size differed significantly between regions. In the Hainich size of thorax was significantly smaller and size of eyes was significantly bigger compared to the Schwäbische Alb.
[2)] In the Schwäbische Alb, but not in the Hainich, relative leg length increased with land-use intensity.
[3)] In the Schwäbische Alb variation in femur length was smaller than in Hainich.

The relative size of the head decreased with land-use intensity. Mowing frequency and fertilization intensity per se had a negative effect on relative size of head, whereas grazing intensity did not significantly affect the size of heads (Fig. 4.5). Relative antenna length did not change with land use, despite of the significant negative effect of mowing frequency on antenna length. Eye size and mandible length did not change with land-use intensity and these patterns were equal for all land use components (Fig. 4.5). Proboscis length decreased with land-use intensity due to significant negative effects of mowing and fertilization. There was no significant effect of grazing intensity on relative proboscis length. Relative wing area decreased with land-use intensity driven by negative effects of

mowing, while grazing and fertilization intensity had no significant effect on wing area. Relative leg length increased with mowing frequency but was not related to grazing intensity and fertilization intensity (Fig. 4.5). Relative femur length did not change with overall land-use intensity but was positively related to mowing frequency and negatively to grazing intensity. Hairiness decreased with land-use intensity, because of the negative effects of mowing and fertilization on hairiness. Grazing intensity seemed to have a positive effect on hairiness.

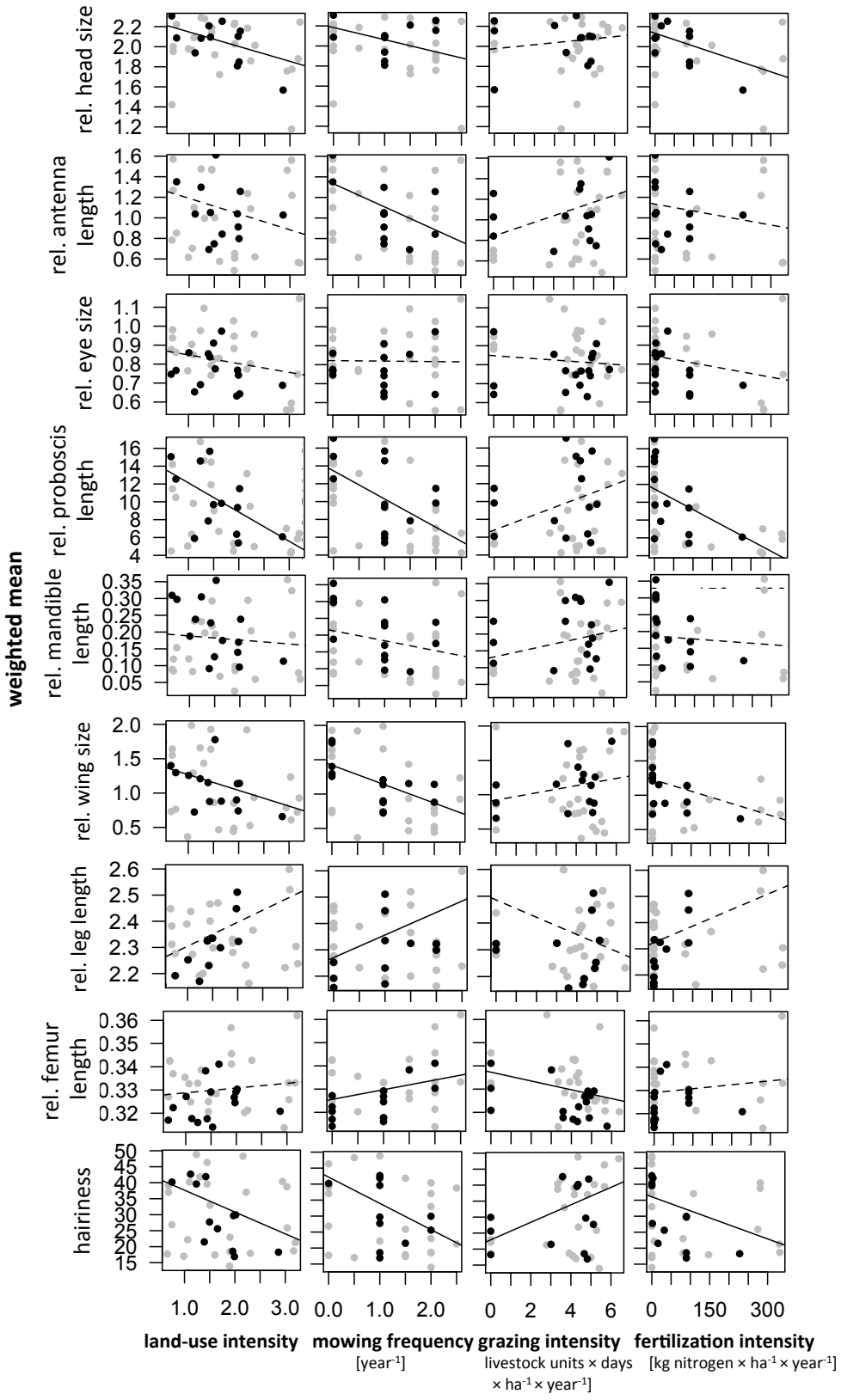


Fig. 4.5: Changes of mean relative size of body parts (community-weighted means) with land-use intensity (averaged for 2011 and 2012). In addition to the compound land-use intensity (LUI) index, mowing frequency, log grazing intensity and fertilization intensity were analyzed separately. Different regions are indicated by different colors (Hainich: grey, Schwäbische Alb: black). Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships.

The variability of traits (coefficient of variation) also changed significantly with land-use intensity for the relative size of the head, wing area, eye size and for hairiness. Effects of land-use intensity on variation in hairiness were only found in the Schwäbische Alb but not in the Hainich (Table 4.2).

Variation of community weighted means of head size increased with land use intensity due to positive effects of mowing frequency and fertilization intensity, whereas grazing intensity had no significant effect on head size (Fig. 4.6). Fertilization intensity had a positive effect on eye size, whereas mowing frequency and grazing intensity had no significant effects on eye size. Coefficient of variation of relative wing size decreased with land use intensity, especially with mowing and fertilization. Grazing intensity had no significant effect on variation of wing size (Fig. 4.6). Variation in hairiness did not change with land use due to oppositional effects of mowing (positive) and grazing (negative). Variation of community weighted means of antenna length, proboscis length, mandible length, leg length and femur length did not change significantly with land-use intensity or specific land-use components.

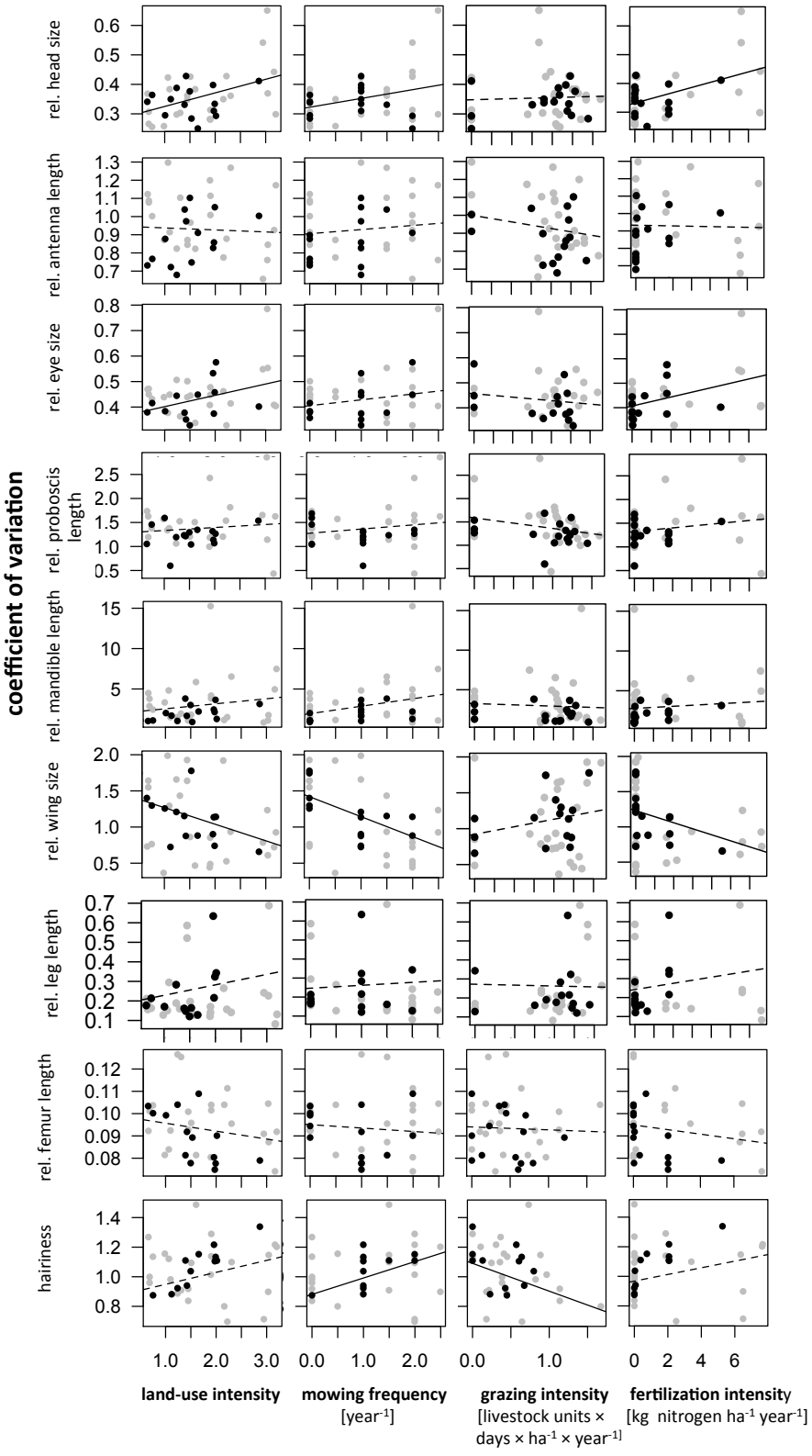


Fig. 4.6: Linear regressions of community weighted coefficients of variation (CV) of traits depending on land-use intensity (averaged for 2011 and 2012). Additionally, components of land use intensity (mowing frequency, grazing intensity (log) and fertilization intensity) were analyzed separately. Different regions are indicated by different colors (Hainich: grey, Schwäbische Alb: black). Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships.

Differences between orders

Morphological traits differed significantly across insect orders (Fig. 4.7). Hymenopterans and butterflies were bigger than beetles and flies. The smallest heads where those of beetles, followed by heads of flies. Hymenopterans and butterflies had the smallest heads. Flies had the shortest antenna; hymenopterans and beetles were intermediate and butterflies had the longest antenna. Beetles had smallest eyes followed by hymenopterans and butterflies. Flies had the biggest eyes. Proboscides of hymenopterans were longer than proboscides of flies and beetles (only few individuals with proboscis). Longest proboscides where those of butterflies. There were just two insect orders with mandibles. Hymenopterans had significantly longer mandibles than beetles. Relative wing area of beetles, flies and hymenopterans were smaller than the much bigger relative wing area of butterflies. Hymenopterans had the shortest femora, compared to longer femora of other orders. Flies and butterflies had significantly longer legs as beetles and hymenopterans. Many flies were nearly hairless, and also beetles were rather slightly hairy. Hymenopterans where more hairy, and butterflies were nearly completely covered by hairs.

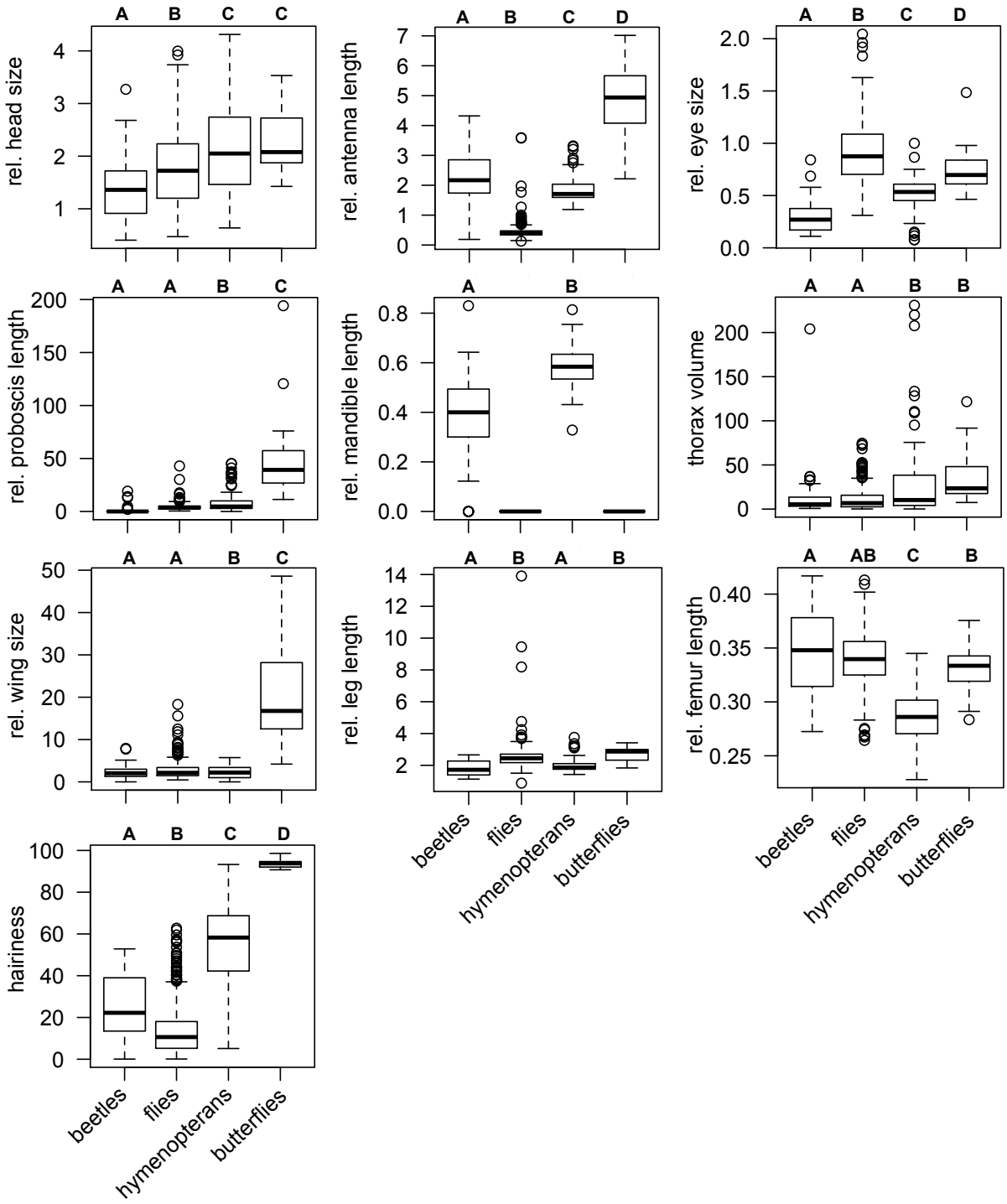


Fig. 4.7: Differences of community weighted mean traits within insect orders. Unequal letters indicate significant differences.

Differences within orders

To investigate whether changes in traits with land use also occurred among closely related species, we repeated the above analyses for subsets of the community, including only species from the same insect

order. We found significant changes of mean traits with land-use intensity within orders in several cases (Fig. 4.8).

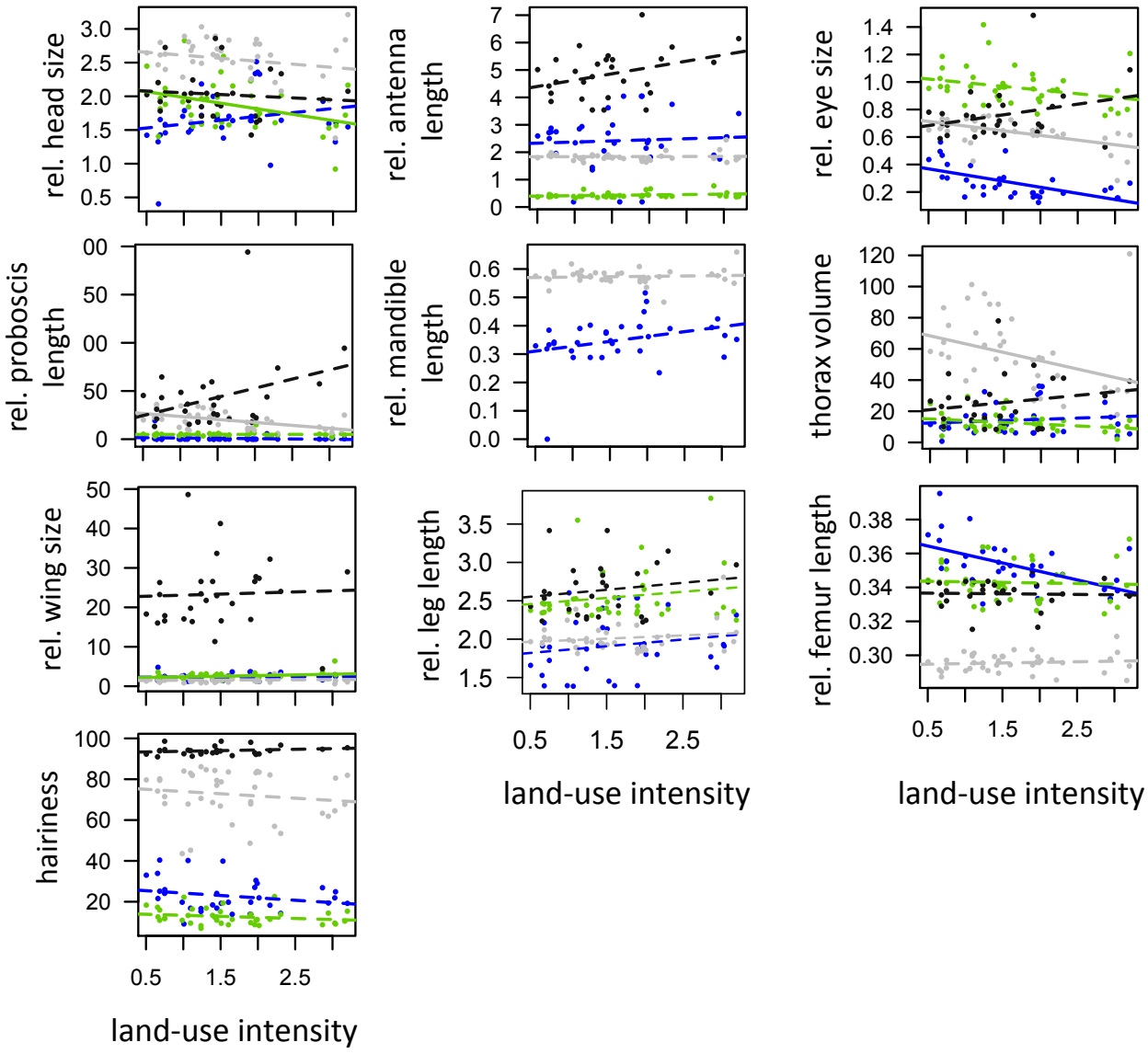


Fig. 4.8: Effects of land-use intensity on community weighted mean traits of pollinator communities, shown for each insect order (beetles: blue, flies: green, hymenopterans: grey, butterflies: black). Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships.

The head size decreased significantly with land-use within flies, but not within the other insect orders. The proboscis length of hymenopterans decreased significantly with land-use intensity but not the other orders. The body size (thorax volume) decreased significantly with land-use intensity within hymenopterans, but not within beetles, flies and butterflies. The relative wing area of flies increased

significantly with land use intensity, but with a very low slope, and the other orders showed no effect. Relative femur length decreased with land- use intensity only within beetles. Relative length of antennas, mandibles and legs as well as and hairiness did not change with land-use intensity within insect orders.

Discussion

The traits of pollinators were clearly associated with the habitat in which they occur: mean morphological traits of pollinator communities shifted with land-use intensity. Although changes in community-weighted means were prominent, trait ranges did not become narrower with increasing land-use intensity.

Effects of land-use intensity are often evaluated by investigating changes in species diversity. Species diversity may largely change in concert with trait diversity (Flynn *et al.* 2009). In contrast, our study shows strong effects of land-use intensity on pollinator traits, although species diversity is unchanged. Most of the selected traits, and also all of them together (PCA), showed responses to land use, probably due to taxonomic shifts in the communities. As expected, the four insect orders differed strongly in trait space. Nevertheless, the shifts with land-use intensity were still strong when the percentage of flies – the major taxonomic change in communities with land use – was factored out.

In several cases individual land-use components could be identified as potential drivers for these trends. Fertilization and mowing intensity explained all trait shifts with land-use intensity in general, except changes in relative wing size that were not related to fertilization. Whereas fertilization and mowing were positively correlated, high grazing intensity was often associated with little or no fertilization and with infrequent or no mowing (Blüthgen *et al.* 2012). Thus, mowing frequency and grazing intensity mostly showed contrasting trends for each trait. Grazing seems to be less important since only hairiness was significantly related to grazing.

To predict and understand potential functional consequences of shifts in trait composition to the ecosystem, it is important to examine the relevance of certain traits on the ecology, behavior or physiology of an individual and its taxonomic variability. Body size – a trait that decreased by half with land-use intensity in our study – is considered as the most important trait of an animal and predicts for example its metabolic rate (Brown 1995). Independent body length measures for the same communities confirmed this negative trend (Kühsel & Blüthgen 2015). Body size is also correlated with

foraging flight distance (Gathmann & Tscharntke 2002; Greenleaf *et al.* 2007) and can be expected to strongly influence the scale over which insects can access resources, except when passive drift comes into play at very small body sizes. Especially the size of the thorax could be an indicator of mobility, as its size relates to the size of flight muscles (Dudley 2002). Smaller, less mobile bees, might therefore be uncommon if required nesting habitats can not be found in the immediate vicinity (Westrich 1990). Furthermore, a small body size implies a high surface to volume ratio and, therefore, could indicate higher water loss (Addo-Bediako, Chown & Gaston 2001), and thus a higher dependence on water availability and limit the species' thermal niche. Indeed, thermal activities of smaller pollinator species have an optimum at lower temperatures than large-bodied species (Kühsel & Blüthgen 2015). Furthermore, extreme winter warming events are known to more negatively impact small rather than large soil fauna (Bokhorst *et al.* 2012) which was explained by traits and not by taxa. From another point of view, small-bodied species require fewer resources to produce offspring than do large-bodied species and so may be better able to survive in disturbed or degraded habitats (Williams *et al.* 2010). Small pollinators, which were especially flies in our study, may profit from this shift in body size and indeed, the proportion of flies on the pollinator community was found to increase with land-use intensity.

In addition to body size, relative wing size decreased with land-use intensity, corresponding to a decreasing proportion of large winged butterflies. Wing size is also important in order to predict species' dispersal ability. In turn, mobility can be used to predict responses to habitat fragmentation and agricultural intensification (Thomas 2000; Gabriel *et al.* 2010). In structurally poor, intensively used grasslands distance between food resources and nesting habitats is expected to be larger than in more heterogeneous grasslands (Steffan-Dewenter 2002; Willimas & Kremen 2007), a disadvantage for species with limited mobility.

Hairiness of pollinator species at higher land-use intensities was reduced by 45%. Pronounced hairiness can protect insects from heat and strong solar radiation but can also serve as protection against cooling and thus make pollinators more tolerant to different weather conditions (Kevan, Thomas & Shorthouse 1982). Less hairy animals such as flies in intensively used grasslands thus could be more susceptible to adverse weather conditions compared to more hairy pollinators such as butterflies and bees. Both butterflies and bees had higher thermal optima than other pollinator taxa (Kühsel & Blüthgen 2015), and their proportion in the community decreased with land use. The decrease in butterflies and bees also contributed to the average decline of proboscis length with land-use intensity. Long proboscides often imply a specialization in certain food plants (Müller 1995). Plant species diversity is known to strongly decrease with land-use intensity (Weiner *et al.* 2014; Kühsel &

Blüthgen 2015). Plant species remaining in intensively used sites, dominated by Asteraceae (Weiner *et al.* 2014; Kühsel & Blüthgen 2015), have open, easily exploitable flowers and thus mainly serve as food resource for pollinators with short proboscides – and may thus be one of the drivers of the changes in pollinator community composition.

Furthermore, leg length that could also be an indicator for mobility and antenna length that is important for orientation at larger scales did not change consistently with land-use intensity in this study. The trend to shorter legs and shorter antenna was related to mowing intensity.

These findings confirm hypotheses of previous studies: environmental filtering is a fundamental process influencing community assembly. For example, functional traits and environmental filtering were identified as drivers of community assembly in a species-rich tropical system (Lebrija-Trejos *et al.* 2010). Morphological traits of ant communities were as important as species identity to assess differences in community structures among land-use types (Yates & Andrew 2011).

Land-use intensity in grasslands promoted flies – either because of the flies' typical traits, or the typical traits became more common because flies became dominant for other reasons (Kühsel and Blüthgen 2015). The flies' smaller bodies and weak hairiness can provide disadvantages in terms of mobility and physiology. However, their short proboscides can be beneficial on intensively used grasslands. But it is largely unknown, if pollination in temperate grassland ecosystem can be made by flies alone.

Variability in traits between species was largely unchanged across the land-use intensity gradient, and only showed a significant trend in few cases. Variability in head size and relative eye size even increased with land use, although species diversity did not increase. Correspondingly, our study on thermal niches showed an increase in the diversity of thermal niches with land-use intensity, suggesting an increase in the resilience of communities against environmental variability (Kühsel and Blüthgen 2015). This stabilization through higher diversity of response traits (Elmqvist *et al.* 2003) may thus extend to several traits and possible functions. A community with larger trait heterogeneity could thus be an advantage in contrast to a more monotonous one that could lead to a pollinator lack for some plants and/or at unfavorable conditions.

The traits are not only affected by land use, but also differed systematically between insect orders. For example, the butterflies had the highest relative wing and proboscis size. Since we know that the traits depend on taxonomic affiliation, phylogenetic relationships of the species can also be informative for the effects of land-use intensity. As no phylogenetic tree exists for this large spectrum of different species, we instead investigated if trait changes with land-use also occur within each insect order. If

this was the case, not only the proportional changes of insect orders would be responsible to shifts in the expression of traits, but also the species composition within a taxon. In most cases, the average traits did *not* change within an order. Only for certain traits, within-taxon trends with land-use intensity were found, suggesting that changes in the relative composition of the four taxa are partly responsible for the effects. The proportion of flies alone, however, although the most prominent taxonomic change, did not explain these shifts, as the general findings were unaffected when controlled for this variable.

Changes in community structure can be partly predict with functional traits (Lavorel & Garnier 2002; Webb *et al.* 2010), but a range of other processes (e.g. competition or predation) can also generate patterns of species segregation along environmental gradients (Gotelli & McCabe 2002; Hausdorf & Hennig 2007; Englund *et al.* 2009).

Our results strongly support the idea that changes in the composition of pollinator functional traits are a more sensitive indicator for land-use intensity, and that traditional measures of species diversity alone can fail to detect land use responses (Brown 1997). As an addition to monitoring programs of species richness and species composition, traits allow to better link environmental drivers to processes controlling community dynamics. The ability to predict the responses of ecological communities and individual species to human-induced environmental change remains a key issue for ecologists and conservation managers alike (Larsen, Williams & Kremen 2005).

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Supplementary Information

Supplementary Table 4.1: List of species with geometric mean of the thorax volume (length × width × height, T_{geo}), number of plots were the species were found (no. of localities), and the sex of the measured individual.

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Adia cinerella*	1.82	9	female
	Allophorocera ferruginea	2.77	2	female
	Angioneura fimbriata	1.54	1	male
	Anthomyia liturata	1.98	21	female
	Azelia trigonica	1.02	1	female
	Bellardia bayeri	2.82	1	male
	Bellardia pandia	3.55	6	female
	Bellardia viarum	3.02	2	undetermined
	Bellardia vulgaris	2.83	13	undetermined
	Bibio johannis	1.75	2	male
	Bibio leucopterus	2.49	1	female
	Bicellaria spuria	0.86	3	female
	Bicellaria sulcata	0.81	1	female
	Billaea triangulifera	3.33	1	female
	Bithia spreta	2.46	2	female
	Blaesoxipha laticornis	2.33	2	female
	Blaesoxipha plumicornis	2.28	1	male
	Blondelia nigripes	2.79	5	female
	Bombylius ater	2.06	1	female
	Bombylius major	3.41	1	female
	Bombylius venosus	3.51	3	male
	Botanophila biciliaris	2.03	9	female
	Botanophila brunneilinea	2.31	4	female
	Botanophila discreta	1.90	5	female
	Botanophila fugax	1.87	26	female
	Botanophila seneciella	1.61	1	male
	Botanophila striolata	1.84	4	female
	Botanophila varicolor	2.32	2	female
	Brachicoma devia	2.79	2	undetermined
	Calliphora vicina	3.80	2	undetermined
	Calliphora vomitoria	4.22	1	undetermined
	Calythea nigricans	1.64	5	female
	Cheilosia albitarsis	3.24	6	female
	Cheilosia barbata	2.78	4	female
	Cheilosia canicularis	4.63	4	female
	Cheilosia gigantea	3.16	1	male
	Cheilosia impressa	2.06	1	female
	Cheilosia lenis	3.06	1	female
	Cheilosia pagana	2.26	2	female
	Cheilosia ranunculi	3.15	1	male
	Cheilosia scutellata	2.91	1	male
	Cheilosia soror	3.22	4	male
	Cheilosia vernalis	2.36	10	female
	Cheilosia vulpina	3.43	5	female
	Chloromyia formosa	2.11	10	undetermined
	Chlorops pumilionis	1.15	2	female
	Chrysogaster basalis	2.44	1	male
	Chrysogaster solstitialis	2.48	2	female
	Chrysotoxum arcuatum	4.14	1	female
	Chrysotoxum bicinctum	3.32	5	female
	Chrysotoxum festivum	4.06	6	female
	Chrysotoxum vernale	3.83	1	male
	Chyliza extenuata	1.79	1	undetermined
	Coenosia infantula	1.24	6	undetermined
	Coenosia pumila	1.25	1	female
	Coenosia tigrina	2.05	8	female

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Cynomya mortuorum*	3.97	21	female
	Dasysyrphus albostriatus	3.10	1	male
	Dasysyrphus hilaris	2.91	1	female
	Dasysyrphus pinastri	2.74	1	female
	Dasysyrphus tricinctus	3.04	1	female
	Delia coarctata	1.93	1	female
	Delia florilega	1.39	12	female
	Delia lophota	2.12	11	female
	Delia platura	1.45	27	female
	Delia radicum	2.09	14	female
	Dexia rustica	3.75	1	male
	Dilophus febrilis	1.83	14	female
	Dilophus femoratus	1.47	3	female
	Dilophus humeralis	1.21	2	male
	Dinera ferina	3.75	2	female
	Diplonerva nitidula	0.81	2	female
	Dolichopus ungulatus	1.84	1	undetermined
	Drino vicina	2.68	1	female
	Ecoptomera pallescens	1.68	1	undetermined
	Eggisops pecchiolii	2.08	1	male
	Eloceria delecta	1.43	1	male
	Empis aestiva	0.95	1	female
	Empis caudatula	1.37	14	female
	Empis ciliata	2.81	2	male
	Empis dimidiata	2.01	3	female
	Empis femorata	1.89	1	male
	Empis grisea	1.84	1	female
	Empis laminata	1.09	1	male
	Empis lepidopus	1.57	3	undetermined
	Empis livida	2.58	26	female
	Empis nuntia	1.00	3	female
	Empis opaca	2.63	2	female
	Empis pseudonuntia	1.42	6	female
	Empis tesselata	3.20	11	female
	Epicampocera succinata	2.65	3	male
	Episyrphus balteatus	2.80	29	female
	Eriothrix rufomaculata	2.69	5	female
	Eristalinus sepulchralis	3.39	1	female
	Eristalis abusiva	3.47	2	undetermined
	Eristalis arbustorum	3.12	10	female
	Eristalis horticola	3.87	1	female
	Eristalis nemorum	4.32	7	female
	Eristalis similis	4.57	1	female
	Eristalis tenax	4.55	27	female
	Estheria cristata	3.66	3	male
	Eudasyphora cyanicolor	3.09	6	female
	Eumerus strigatus	2.20	1	female
	Eupeodes corollae	2.61	25	female
	Eupeodes latifasciatus	2.47	4	female
	Eupeodes luniger	2.59	6	female
	Eurithia connivens	3.21	1	male
	Eurychaeta palpalis	3.52	4	undetermined
	Exorista mimula	1.99	1	male
	Exorista rustica	2.82	4	female
	Exorista tubulosa	2.45	2	male
	Fannia armata	1.66	3	female
	Fannia canicularis	2.06	1	female
	Fannia latipalpis	1.83	5	female
	Fannia postica	1.63	1	female
	Fannia rondanii	1.20	2	female
	Fannia serena	1.37	15	female
	Fannia sociella	1.55	2	female

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Fannia umbrosa*	1.48	3	female
	Geomyza tripunctata	1.01	1	male
	Gonia capitata	4.44	1	female
	Gonia geniculata	4.36	1	female
	Graphomya maculata	3.17	2	female
	Haematopota pluvialis	2.94	2	female
	Hebecnema nigra	1.68	1	male
	Hebecnema umbratica	1.79	1	male
	Hebecnema vespertina	1.64	1	female
	Helina impuncta	2.89	5	female
	Helina latitarsis	2.17	9	female
	Helina laxifrons	2.33	2	female
	Helina quadrum	2.60	2	female
	Helina reversio	1.92	15	female
	Helina trivittata	2.44	10	female
	Helophilus hybridus	4.37	3	female
	Helophilus pendulus	3.75	5	female
	Helophilus trivittatus	4.09	8	female
	Heterostylodes nominabilis	1.11	6	female
	Heterostylodes obscurus	1.33	3	female
	Hilara longivittata	0.98	3	male
	Hybomitra ciureai	4.09	1	male
	Hydrophoria silvicola	1.93	1	male
	Hydrotaea albipuncta	1.62	1	female
	Hydrotaea dentipes	2.60	1	female
	Hydrotaea meteorica	1.84	1	female
	Hydrotaea pandellei	1.88	1	female
	Hydrotaea parva	1.19	2	female
	Hylemya partita	1.58	1	female
	Hylemya urbica	1.93	16	female
	Hylemya variata	2.02	9	female
	Leptogaster cylindrica	1.75	1	female
	Limnia unguicornis	1.58	4	female
	Linnaemya picta	3.98	4	female
	Loewia phaeoptera	1.33	1	female
	Lonchoptera bifurcata	0.71	1	undetermined
	Lophosceles cinereiventris	1.72	1	female
	Lucilia caesar	3.19	7	undetermined
	Lucilia illustris	2.98	6	undetermined
	Lucilia sericata	3.00	2	undetermined
	Lucilia silvarum	3.31	26	female
	Lydella stabulans	2.21	1	male
	Machimus rusticus	4.65	1	undetermined
	Macquartia praefica	2.85	3	female
	Meigenia dorsalis	1.98	1	male
	Meigenia uncinata	2.17	2	male
	Melangyna labiatarum	2.21	2	female
	Melanogaster nuda	1.78	5	female
	Melanomya nana	1.29	3	undetermined
	Melanostoma mellinum	1.66	33	female
	Melanostoma scalare	1.99	2	female
	Melinda gentilis	2.30	8	undetermined
	Melinda viridicyanea	2.91	7	undetermined
	Meliscaeva cinctella	2.69	2	female
	Merodon equestris	5.20	1	female
	Meromyza femorata	1.23	3	female
	Mesembrina meridiana	4.63	1	female
	Microchrysa flavicornis	1.50	1	male
	Microsoma exiguum	1.63	1	male
	Morellia aenescens	2.40	9	female
	Morellia hortorum	2.76	3	female
	Morellia podagrica	2.72	1	female

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Morellia simplex*	2.57	2	female
	Musca autumnalis	2.15	12	female
	Musca domestica	2.48	1	male
	Musca osiris	2.09	1	male
	Muscina levida	2.44	2	female
	Muscina pascuorum	3.41	3	female
	Myathropa florea	4.53	12	female
	Mydaea nebulosa	2.66	1	male
	Myospila meditabunda	2.36	6	female
	Neomyia cornicina	2.74	12	female
	Neomyia viridescens	3.31	14	female
	Nephrotoma appendiculata	2.12	7	female
	Nephrotoma flavescens	1.84	4	male
	Nilea rufiscutellaris	2.94	1	female
	Nowickia ferox	4.64	5	female
	Nyctia halterata	1.91	1	female
	Ocytata pallipes	2.56	2	male
	Onesia floralis	3.17	8	female
	Opesia cana	2.62	2	female
	Ophiomyia nasuta	0.94	4	undetermined
	Opomyza germinationis	1.28	1	female
	Orellia falcata	2.06	5	female
	Oscinella frit	0.70	4	female
	Oxyna flavipennis	1.55	6	female
	Pales pavida	2.86	3	female
	Paracraspedothrix montivaga	1.03	1	male
	Paragus haemorrhous	1.43	1	male
	Paramacronychia flavipalpis	3.68	8	female
	Parasyrphus annulatus	2.22	4	female
	Pegomya meridiana	1.22	1	male
	Pegoplata aestiva	1.81	19	female
	Pegoplata infirma	1.34	3	female
	Pegoplata nigroscutellata	1.84	2	female
	Pelecocera tricincta	1.25	2	female
	Phaonia angelicae	3.21	14	female
	Phaonia consobrina	3.20	1	female
	Phaonia meigeni	2.90	3	female
	Phaonia pallida	2.50	2	female
	Phaonia serva	3.19	3	female
	Phasia hemiptera	3.65	7	female
	Phasia obesa	1.89	13	female
	Phasia pusilla	1.45	3	female
	Pherbellia cinerella	1.62	1	female
	Pherbellia griseola	1.27	2	female
	Phorbia bartaki	1.33	1	male
	Phorbia genitalis	1.30	2	undetermined
	Phorbia juncorum	1.37	1	male
	Phorbia unipila	1.34	2	female
	Phryxe heraclei	2.55	2	female
	Physiphora alceae	1.72	1	undetermined
	Phytomyza albipennis	1.04	7	undetermined
	Pipiza noctiluca	2.64	4	female
	Pipiza quadrimaculata	1.51	1	female
	Pipizella viduata	1.65	13	female
	Platycheirus albimanus	1.69	27	female
	Platycheirus clypeatus	1.97	9	female
	Platycheirus europaeus	2.09	2	female
	Platycheirus manicatus	2.57	7	female
	Platycheirus peltatus	2.63	3	female
	Platycheirus tarsalis	2.07	1	female
	Platymya fimbriata	2.24	2	female
	Platypalpus agilis	0.96	1	female

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Platypalpus albiseta*	0.79	1	male
	Platypalpus cruralis	1.42	2	female
	Platystoma seminationis	1.99	3	male
	Polietes lardarius	3.41	3	female
	Pollenia amentaria	3.54	18	undetermined
	Pollenia angustigena	2.55	4	undetermined
	Pollenia hungarica	2.52	8	undetermined
	Pollenia labialis	2.93	2	undetermined
	Pollenia pediculata	2.36	29	undetermined
	Pollenia rudis	2.22	12	undetermined
	Prosena siberita	3.00	3	female
	Protocalliphora falcozi	3.54	1	male
	Protocalliphora peusi	3.62	1	female
	Protophormia terraenovae	3.45	1	undetermined
	Psila merdaria	2.49	1	female
	Pyrellia rapax	1.91	11	female
	Ravinia pernix	2.45	3	female
	Rhagio scolopaceus	2.48	4	female
	Rhagio strigosus	3.17	1	female
	Rhamphomyia longipes	0.90	1	female
	Rhamphomyia sulcata	1.33	3	female
	Rhingia campestris	3.44	4	female
	Rhinomorinia sarcophagina	2.25	4	female
	Saltella sphondylii	1.32	3	female
	Sarcophaga aratrix	2.92	1	female
	Sarcophaga bulgarica	2.69	2	male
	Sarcophaga caerulescens	5.07	1	undetermined
	Sarcophaga carnaria	3.28	35	female
	Sarcophaga crassimargo	2.18	4	undetermined
	Sarcophaga depressifrons	2.23	2	undetermined
	Sarcophaga incisilobata	2.47	13	undetermined
	Sarcophaga jeanleclercqi	3.40	1	female
	Sarcophaga melanura	3.12	3	undetermined
	Sarcophaga nemoralis	2.26	2	female
	Sarcophaga noverca	3.09	2	female
	Sarcophaga pumila	1.84	7	female
	Sarcophaga schineri	3.59	3	male
	Sarcophaga subvicina	4.28	22	male
	Sarcophaga vagans	3.54	4	undetermined
	Sarcophaga variegeta	3.60	36	female
	Sarcophaga vicina	3.26	1	undetermined
	Scaeva pyrastri	3.55	18	female
	Scaeva selenitica	3.72	1	female
	Scaptomyza graminum	0.85	1	female
	Scathophaga stercoraria	2.89	6	female
	Schwenckfeldina carbonaria	0.88	2	female
	Sciaria flavimana	0.93	3	female
	Sepsis cynipsea	1.06	5	female
	Sepsis duplicata	0.69	1	female
	Sepsis thoracica	1.07	2	female
	Sicus ferrugineus	2.59	1	female
	Siphona geniculata	1.85	12	female
	Solieria pacifica	2.27	7	female
	Sphaerophoria interrupta Gruppe	1.88	33	female
	Sphaerophoria taeniata	1.98	22	undetermined
	Sphaerophoria scripta	2.19	33	female
	Strongygaster globula	1.95	2	female
	Synathica parvula	1.70	1	female
	Siphonella oscinia	0.85	1	female
	Syritta pipiens	2.32	13	female
	Syrphus ribesii	3.32	13	female
	Syrphus torvus	2.90	3	female

order	species	T_{geo}	no. of localities	sex of measured individual
Diptera	*Syrphus vitripennis*	3.05	18	female
	Tachina fera	4.08	5	female
	Tachina magnicornis	4.49	5	female
	Tephritis crepidis	1.42	1	female
	Thecophora atra	1.66	3	female
	Thecophora distincta	1.72	5	female
	Thecophora fulvipes	1.33	3	male
	Thereva valida	3.49	1	male
	Thricops cunctans	2.16	2	female
	Thricops longipes	2.81	8	female
	Thricops nigrifrons	2.37	10	female
	Thricops semicinereus	2.03	6	female
	Tipula vernalis	3.04	5	female
	Tolmerus atricapillus	3.19	3	female
	Toxoneura quinquemaculata	1.53	3	female
	Trachysiphonella scutellata	0.89	2	female
	Trichactia pictiventris	2.07	1	female
	Trixa conspersa	3.50	1	male
	Volucella bombylans	5.22	1	female
	Volucella pellucens	5.17	3	female
	Voria ruralis	2.44	3	female
	Winthemia quadripustulata	3.31	1	male
	Xanthogramma pedissequum	2.96	3	female
Hymenoptera	*Andrena agilissima*	4.41	1	female
	Andrena anthrisci	1.74	1	female
	Andrena bicolor	2.97	1	undetermined
	Andrena carantonica	4.16	1	female
	Andrena chrysosceles	2.56	10	undetermined
	Andrena cineraria	4.25	2	female
	Andrena combinata	3.14	1	female
	Andrena dorsata	2.65	2	female
	Andrena flavipes	3.44	11	undetermined
	Andrena florivaga	3.00	1	female
	Andrena fucata	3.21	1	undetermined
	Andrena fulva	4.26	1	female
	Andrena fulvago	2.53	3	female
	Andrena fulvicornis	2.46	2	female
	Andrena gravida	4.19	1	female
	Andrena haemorrhoa	3.12	6	female
	Andrena minutula	2.03	4	female
	Andrena nigroaenea	3.97	5	undetermined
	Andrena nitida	4.44	4	female
	Andrena nitidiuscula	2.53	1	female
	Andrena potentillae	2.08	1	female
	Andrena proxima	2.74	1	undetermined
	Andrena semilaevis	2.04	2	undetermined
	Andrena subopaca	1.69	5	undetermined
	Andrena tibialis	4.21	1	female
	Andrena varians	4.34	1	female
	Apis mellifera	3.92	32	female
	Athalia rosae	2.40	9	female
	Bombus bohemicus	6.34	7	female
	Bombus hortorum	5.67	9	female
	Bombus humilis	4.65	10	female
	Bombus lapidarius	6.26	35	female
	Bombus lucorum agg	4.97	19	female
	Bombus pascuorum	5.93	25	female
	Bombus pratorum	4.19	5	female
	Bombus rupestris	7.61	14	female
	Bombus soroeensis	5.95	15	female
	Bombus subterraneus	7.35	1	female
	Bombus sylvarum	4.34	23	female

order	species	T_{geo}	no. of localities	sex of measured individual
Hymenoptera	*Bombus terrestris*	4.75	11	female
	Bombus vestalis	7.49	1	female
	Bombus wurflenii	5.25	11	female
	Cephus pygmeus	1.96	6	female
	Formica cunicularia	1.05	7	female
	Halictus eurygnatus	2.67	2	male
	Halictus rubicundus	3.11	1	female
	Halictus simplex	2.82	5	female
	Halictus tumulorum	1.83	13	female
	Hylaeus annularis	1.75	1	female
	Lasioglossum albipes	2.14	7	undetermined
	Lasioglossum calceatum	2.36	20	female
	Lasioglossum fulvicorne	1.79	12	female
	Lasioglossum laevigatum	2.23	5	female
	Lasioglossum laticeps	2.01	5	female
	Lasioglossum lativentre	2.04	2	undetermined
	Lasioglossum leucopus	1.37	1	female
	Lasioglossum leucozonium	2.41	2	undetermined
	Lasioglossum lineare	1.77	2	undetermined
	Lasioglossum minutulum	1.50	5	undetermined
	Lasioglossum morio	1.39	1	female
	Lasioglossum pauxillum	1.69	19	male
	Lasioglossum villosulum	1.77	4	female
	Lasioglossum zonulum	2.25	1	male
	Lasius emarginatus	0.68	2	female
	Lasius niger	0.84	10	female
	Melitta haemorrhoidalis	3.12	4	undetermined
	Myrmica rubra	0.93	2	undetermined
	Myrmica ruginodis	0.89	4	undetermined
	Nomada flavoguttata	1.67	1	male
	Osmia bicornis	3.36	2	undetermined
	Selandria serva	2.09	8	undetermined
	Sphecodes ephippius	2.26	1	female
	Sphecodes ferruginatus	2.17	2	female
	Sphecodes hyalinatus	1.68	1	female
	Tapinoma ambiguum	0.66	1	undetermined
	Tenthredo atra	3.24	1	undetermined
	Tenthredo notha	3.09	18	undetermined
	Tenthredo temula	3.49	1	undetermined
	Tenthredo vespa	3.37	7	undetermined
	Tiphia femorata	2.16	10	undetermined
	Trachusa byssina	3.66	2	female
Lepidoptera	*Aglais io*	5.35	3	undetermined
	Aglais urticae	4.38	7	undetermined
	Aphantopus hyperantus	3.29	2	undetermined
	Coenonympha glycerion	2.43	2	undetermined
	Erebia aethiops	3.92	1	undetermined
	Hesperia comma	4.35	2	undetermined
	Maniola jurtina	4.56	9	undetermined
	Melanargia galathea	3.58	7	undetermined
	Papilio machaon	6.15	3	undetermined
	Pieris brassicae	4.58	2	undetermined
	Pieris napi	3.02	4	undetermined
	Pieris rapae	3.27	10	undetermined
	Plebeius argus/argyrognomon/ideas agg	2.51	5	undetermined
	Polyommatus bellargus	3.50	1	undetermined
	Polyommatus coridon	3.53	8	undetermined
	Polyommatus icarus	2.52	17	undetermined
	Polyommatus semiargus	2.59	4	undetermined
	Thymelicus lineola	3.36	4	undetermined
	Thymelicus sylvestris	3.23	4	undetermined
	Vanessa cardui	5.59	2	undetermined

order	species	T_{geo}	no. of localities	sex of measured individual
Lepidoptera	*Zygaena carniolica*	3.85	5	undetermined
	Zygaena filipendulae	4.51	2	undetermined
Coleoptera	*Agriotes gallicus*	2.04	1	undetermined
	Agriotes obscurus	3.80	1	undetermined
	Agriotes sputator	1.84	2	undetermined
	Amara familiaris	2.47	1	undetermined
	Anaspis frontalis	1.37	1	undetermined
	Athous bicolor	2.51	3	undetermined
	Athous haemorrhoidalis	3.63	1	undetermined
	Bruchus rufimanus	2.12	1	undetermined
	Cantharis flavilabris	1.85	14	undetermined
	Cantharis fusca	4.12	11	undetermined
	Cantharis livida	3.36	1	undetermined
	Cetonia aurata	7.30	1	undetermined
	Ceutorhynchus assimilis	1.12	2	undetermined
	Chrysanthia geniculata	1.71	3	undetermined
	Cidnopus aeruginosus	3.10	3	undetermined
	Coccinella septumpunctata	3.19	17	undetermined
	Cryptocephalus hypochaeridis	2.53	8	undetermined
	Cryptocephalus populi	1.51	1	undetermined
	Cryptocephalus sericeus	2.96	10	undetermined
	Cryptocephalus vittatus	2.27	1	female
	Dasytes niger	1.14	2	undetermined
	Dasytes plumbeus	1.25	1	undetermined
	Dinoptera collaris	2.69	1	undetermined
	Eusomus ovulum	2.39	1	undetermined
	Glocianus punctiger	1.34	2	undetermined
	Harpalus affinis	3.51	1	undetermined
	Hemicrepidius niger	3.98	6	undetermined
	Hoplia argentea	2.96	5	undetermined
	Lagria hirta	2.00	2	undetermined
	Malachius bipustulatus	1.98	2	undetermined
	Mordellochroa abdominalis	1.86	1	undetermined
	Oedemera femorata	2.06	3	undetermined
	Oedemera lurida	1.53	3	undetermined
	Oedemera virescens	1.90	3	undetermined
	Phyllobius betulinus	2.24	3	undetermined
	Phyllobius pyri	2.18	4	undetermined
	Phyllopertha horticola	4.13	9	undetermined
	Phyllotreta vittula	1.87	4	undetermined
	Poecilus versicolor	3.96	1	undetermined
	Propylea quatuordecimpunctata	2.18	1	undetermined
	Pseudovadonia livida	2.89	6	undetermined
	Rhagonycha fulva	2.27	15	undetermined
	Rhagonycha limbata	1.36	3	undetermined
	Stenurella melanura	2.12	10	undetermined
	Tytthaspis sedecimpunctata	1.73	4	undetermined
	Zacladus geranii	1.38	1	undetermined

Supplementary Table 4.2: Correlations between pollinator diversity ($e^{H'}$) per plot and weighted mean traits and coefficient of variations (CV) of each community (plot). Results are from Spearman rank correlations (N = 40).

	pollinator diversity			
	mean		CV	
community trait	***r***	***p***	***r***	***p***
rel. head size	0.13	0.411	-0.04	0.792
rel. antenna length	-0.06	0.697	0.08	0.625
rel. eye size	0.01	0.954	0.05	0.762
rel. proboscis length	-0.05	0.764	0.03	0.870
rel. mandible length	-0.04	0.82	0.04	0.765
body size (thorax volume)	0.03	0.875	-0.07	0.676
rel. wing area	-0.22	0.172	0.06	0.710
rel. leg length	0.11	0.514	0.15	0.339
rel. femur length	0.04	0.798	-0.15	0.367
hairiness	-0.15	0.358	0.16	0.318

Supplementary Table 4.3: Functional traits and coefficient of variation (CV) of two repeatedly measured species (for each species 5 individuals).

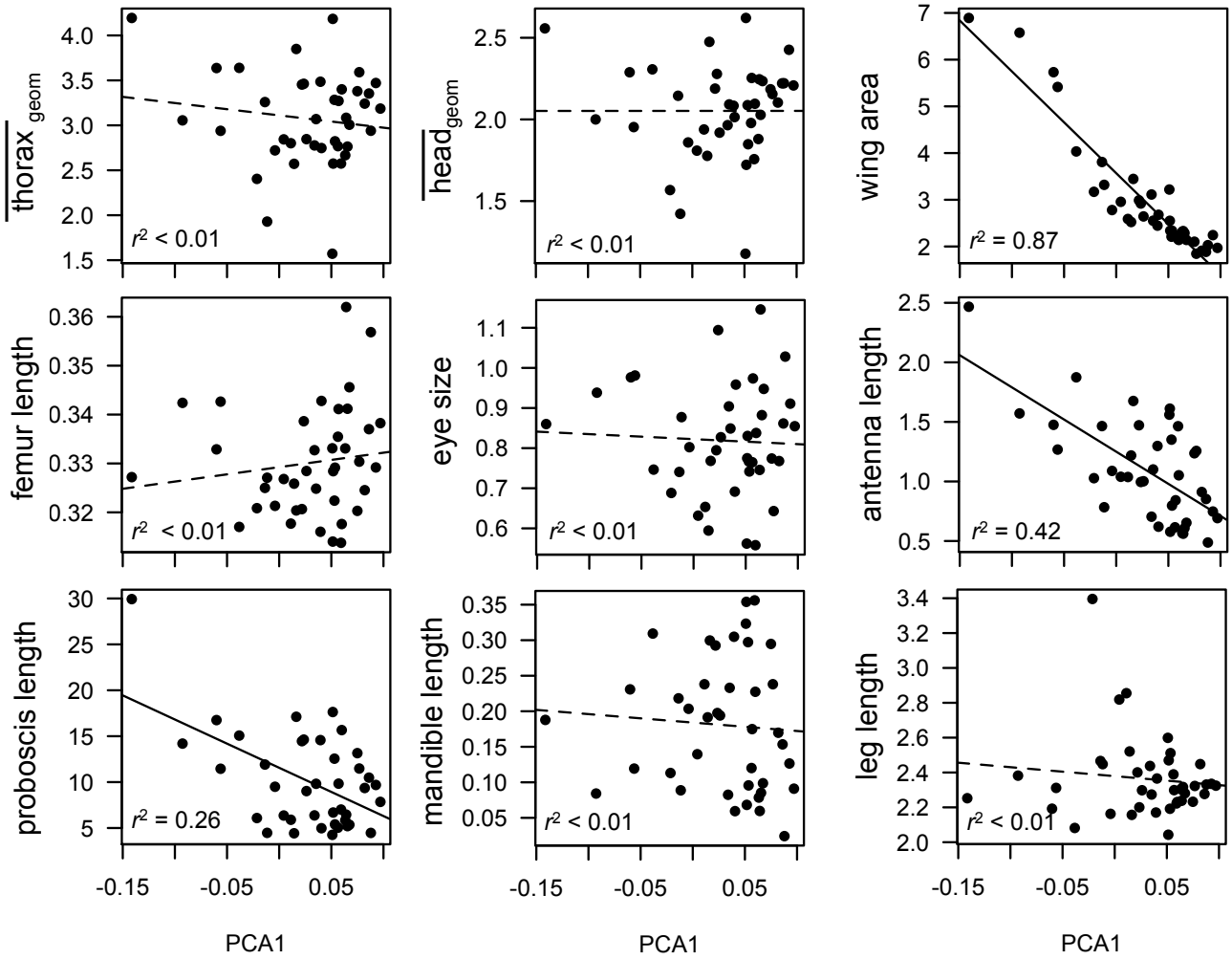
species	rel. head size	rel. antenna length	rel. eye size	rel. proboscis length	rel. mandible length	rel. thorax size	rel. wing size	rel. leg length	hairiness	
Andrena haemorrhoa	2.93	1.33	0.45	na	0.39	2.72	12.33	2.06	39.73	
	2.84	1.50	0.48	na	0.36	2.87	13.15	2.53	18.37	
	2.77	1.36	0.47	0.29	0.42	2.70	12.17	2.00	12.13	
	2.84	1.50	0.49	0.37	0.46	2.77	13.44	2.15	29.38	
	2.97	1.17	0.45	na	0.45	2.91	12.63	2.06	17.18	
$CV_{single\ species}$	0.03	0.10	0.04	0.16	0.10	0.03	0.04	0.10	0.48	
$CV_{single\ species} / CV_{all\ species} \times 100$	6.45	9.18	8.89	8.41	43.60	7.28	2.96	10.02	48.44	mean= 16.9%
Syrphus ribesii	3.00	0.10	1.19	0.30	na	3.19	8.11	1.19	4.45	
	3.43	0.22	1.26	0.28	na	3.58	8.64	2.06	12.73	
	3.25	0.21	1.28	0.27	na	3.44	9.35	1.98	10.62	
	2.34	0.26	0.96	0.20	na	2.46	5.63	1.69	9.23	
	3.57	0.21	1.12	na	na	3.83	9.13	2.03	11.75	
$CV_{single\ species}$	0.16	0.29	0.11	0.17	na	0.16	0.18	0.08	0.33	
$CV_{single\ species} / CV_{all\ species} \times 100$	36.94	26.57	26.61	8.96	na	35.46	12.81	7.80	33.85	mean= 25.9%
CV of all species	0.42	1.10	0.42	1.88	0.23	0.45	1.43	0.10	0.98	

Supplementary Table 4.4: Fixed and interaction effects of land-use intensity index (LUI) and Exploratory (region) on residuals of relative traits (residuals of mean functional trait over the proportion of Diptera on the plots (40 communities). Results are from linear models. Significant values are highlighted in bold.

residuals of trait means	LUI		region		LUI × region	
	F	*p*	*F*	*p*	*F*	*p*
body size (thorax volume)	6.2	**0.018**	3.1	0.085	< 0.1	0.806
head size	5.7	**0.022**	0.8	0.365	1.2	0.280
antenna length	2.8	0.105	0.9	0.344	0.6	0.442
eye size	8.2	**0.007**	2.2	0.149	1.2	0.285
proboscis length	10.6	**0.003**	0.2	0.663	0.1	0.771
mandible length	0.8	0.383	0.3	0.568	0.1	0.721
wing size	5.2	**0.029**	2.9	0.097	0.6	0.443
leg length	3.7	0.060	4.8	**0.036**	11.8	**0.001**
femur length	< 0.1	0.854	2.5	0.126	2.3	0.140
hairiness	9.2	**0.004**	1.7	0.201	1.5	0.223
residuals of trait CVs (coefficient of variation)	LUI		region		LUI × region	
	F	*p*	*F*	*p*	*F*	*p*
body size (thorax volume)	0.7	0.383	7.7	**0.008**	0.1	0.815
head size	12.4	**0.001**	0.6	0.436	0.7	0.398
antenna length	3.1	0.089	1.1	0.306	0.3	0.587
eye size	7.2	**0.011**	4.6	**0.039**	0.9	0.353
proboscis length	0.6	0.448	2.2	0.143	< 0.1	0.854
mandible length	0.3	0.605	0.1	0.724	3.4	0.075
wing size	4.1	0.051	0.3	0.608	0.2	0.664
leg length	2.5	0.125	2.1	0.154	6.1	**0.018**
femur length	1.2	0.288	20.0	**0.003**	< 0.1	0.852
hairiness	2.4	0.128	1.1	0.312	5.4	**0.027**

Mean relative eye size decreased with land-use intensity for residuals of eye size but not for relative eye size itself. There were no effects of region or interaction effects of region and land-use intensity on residuals of community weighted mean traits. Coefficient of variation of wing size and hairiness were significant for relative traits but not for residuals. Residuals of thorax size depended on land-use intensity but not thorax size per se.

Supplementary Fig. 4.1: Pearson's correlations of community weighted means of PCA axis 1 and pollinator species traits. Solid lines indicate significant relationships ($p < 0.05$), dotted lines non-significant relationships.



CHAPTER V

General Discussion

Functional traits and response diversity of pollinators

In this thesis, the influence of land use and varying environmental conditions on functional traits and response diversity of pollinator communities was studied. Pollinator communities were recorded on grassland sites along a land use gradient, and various functional traits (i.e. thermal optima, morphological characteristics) and its variability within the community were determined to examine whether land use has an impact on them. We further investigated thermal niches and water loss of pollinators because they mirror highly relevant responses to changing environmental conditions in the context of climate change.

Different from findings of other studies (Winfree & Kremen 2009; Vanbergen & Initiative 2013) the diversity of pollinators did not decrease with land-use intensity in the study grasslands of the "Biodiversity Exploratories" (Fischer *et al.* 2010, Fig. 5.1a). Although the overall diversity remains unaffected, the composition of the pollinator community changed with land-use intensity. The percentage of butterflies and partially hymenopterans decreased, but was compensated by the increasing proportion of flies (Fig. 5.1b). The single measure of species richness, therefore, is insufficient to assess effects of land-use intensity on ecosystems (Hooper *et al.* 2005; Petchey & Gaston 2006).

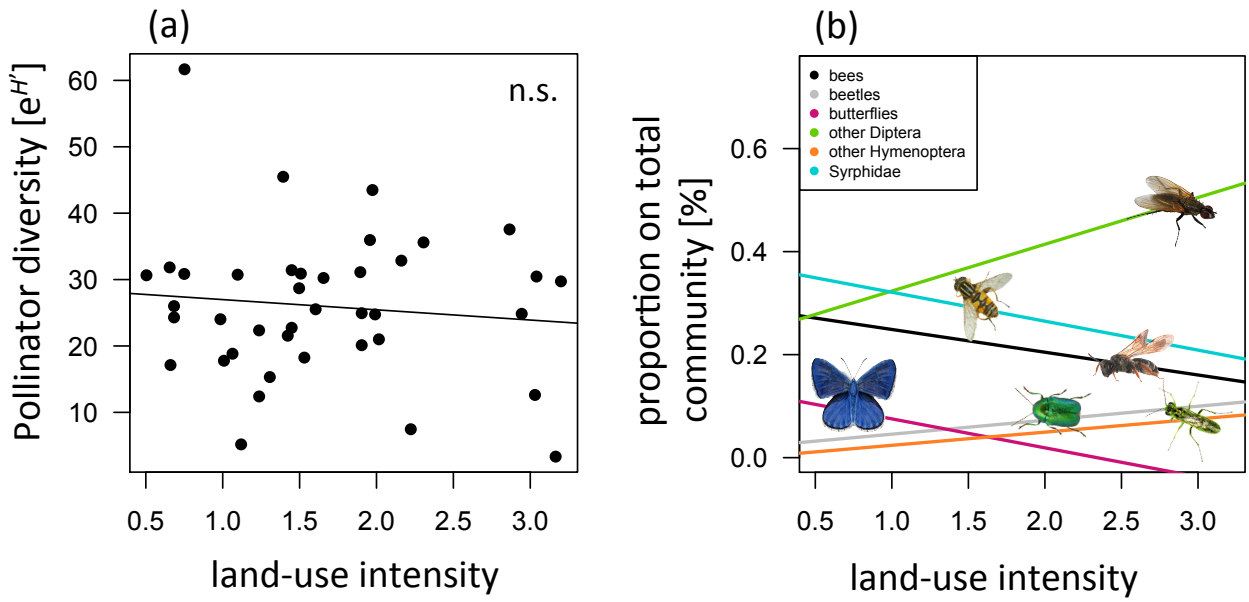


Fig. 5.1: Diversity **(a)** and composition **(b)** of pollinator communities at different land-use intensities (see chapter II for statistical analyses). Pollinator diversity is represented by the exponent of the Shannon's *H'*, weighted by abundance.

We show that the thermal niche breadth and diversity of thermal responses across species together contribute to a higher projected resilience and that land-use intensity per se has no negative effect on thermal niches of pollinators. Species in intensively used grasslands have broader thermal niches and are also more complementary in their thermal optima. Pollinators not only differed in thermal niches but also in water loss. These differences could be significantly explained by the via 3D-models quantified *SA/V* ratios. Although *SA/V* ratios can be predicted to a certain extent by body mass allometries, this do not consider the different forms of insects that also has a significant impact on *SA/V* ratios. Small insects with the largest *SA/V* ratios experienced highest water loss rates.

In addition to land use effects on pollinator thermal niches, body size and several other morphological traits also changed with land-use intensity. However, the trait variability of the community was not negatively affected by land use. Shifts in trait means strongly correspond to shifts in relative abundance of insect orders. Within insect orders consistent land-use changes were rarely found.

Synthesis

Intensive land use and global climate change are considered as threats for the diversity of pollinators (Kremen *et al.* 2007; Potts *et al.* 2010). When species are lost, this can result in serious consequence for the maintenance of ecosystem services (Morandin & Winston 2006; Klein *et al.* 2007). In addition, the effects of diversity, land use or other factors can affect functional traits of species communities (Flynn *et al.* 2009; Laliberté *et al.* 2010). This thesis demonstrates that physiological traits (thermal niches) and morphological traits changed with land-use intensity. Thermal niche breadth and complementarity (response diversity) increased with land-use intensity. Thermal niches are generally very heterogeneous, so that one would expect no narrow community thermal niches in other similarly used grasslands (without extensive habitat destruction), and even at elevated temperatures in a changing climate. It should be noted, however, that flies, which are more common with increasing land use, prefer cooler thermal optima, whereas global warming has the opposite trend.

Land use act as filter also for morphological traits. Community weighted mean traits shift toward smaller body size, lower hairiness and a relatively shorter proboscis and smaller wings. Shifts in trait means mirror the increase in flies, in contrast to the other pollinators, because they most closely match to this morphology. With these pattern, land use can affect not only the number of species, but also the composition of the community (Weiner *et al.* 2014). A shift in traits has possibly also contributed to an increasing commonness of flies with increasing land use, which in turn has an impact on thermal

niches. That the pollinators are smaller at high land-use intensities can also correspond to lower thermal optima. Small animals should lose more water compared to their body size, because of the high surface to volume ratio (Hadley 1994). This makes them more sensitive to high temperatures and dryness (Fung, Lopez & New 2011; New *et al.* 2011; Sanderson 2011), leading to the avoidance of such conditions. Indeed, water loss rates were highest for small pollinator species. To understand differences in water loss rates it is better to directly measure surface area and volume of insects than to predict these with body mass allometry, because measured *SA/V* ratios incorporating body shape can better explain water loss. Water loss rather corresponds to body size than to specific insect orders: smaller animals are more vulnerable to high temperatures and dryness but not: butterflies are more sensitive than flies.

The variability of morphological traits did not decrease and in some cases even increase with land-use intensity. Also our thermal niches showed an increase in the complementarity of thermal niches with land-use intensity, suggesting an increase in the resilience of communities against environmental variability (Kühsel & Blüthgen 2015). This stabilization through a higher diversity of traits (Elmqvist *et al.* 2003) may thus extend to several traits. A community with larger trait heterogeneity could thus be an advantage in contrast to a more monotonous one that could lead to a pollinator lack for some plants and/or at unfavorable conditions.

As the multiple effects of land-use intensity on morphological and physiological traits show, several traits and their variability have to be considered to assess the complex impact of land-use intensity on pollinator communities (Fig. 5.2). From the observation of a single trait, conclusions for the community and the ecosystem are strongly limited. If mean traits and response diversity/variability of traits change, this can affect the ecosystem functioning, which in turn has an impact on ecosystem resilience (Chapin III *et al.* 2000; Elmqvist *et al.* 2003; Mori, Furukawa & Sasaki 2013). We showed that both functional traits and response diversity together can contribute to ecosystem resilience.

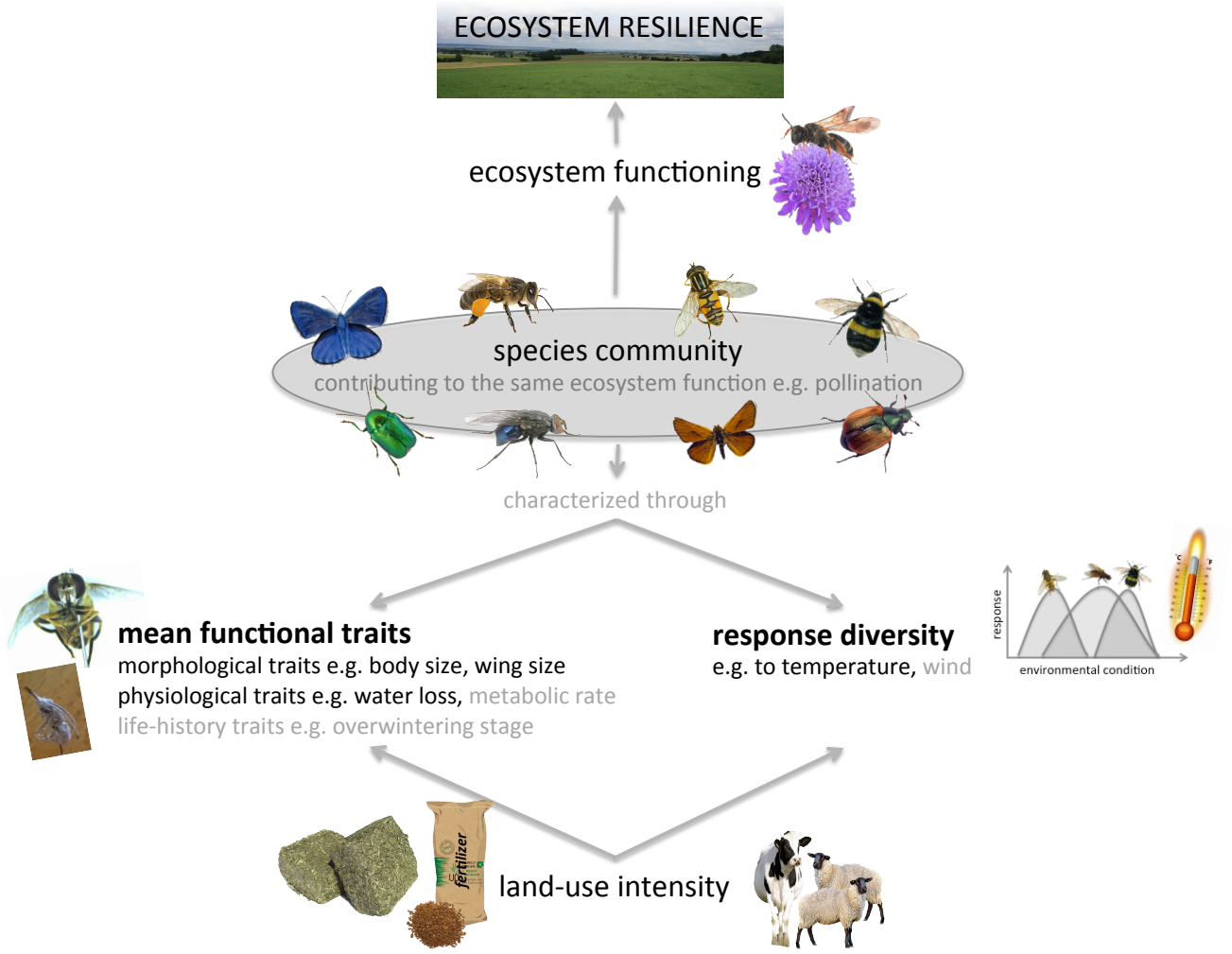


Fig. 5.2: Overview of the contents of this thesis, where all 3 chapters are integrated into an overall context.

Ultimately, it is difficult to decide whether land use affects the traits and thus change the composition of the community, or whether, for example, the decrease in plant species diversity with the increasing land-use intensity remove the floral resources required by some species. This would be probably not a limitation for flies as food generalists that profit from an increasing proportion of Asteraceae with flat flower heads. Pollinators with for example longer proboscides avoid these flowers. Hence, typical fly-traits can become more frequent.

Conclusions

From these results we first conclude that quantifying the diversity of thermal responses of pollinator communities can be very useful to assess the vulnerability of ecosystems to land-use intensification and climate change. Temperature affects pollinator activity as well as physiological aspects of pollinators like water loss. Differences in water loss can in turn lead to a different vulnerability to climate change

that can accompany higher temperatures and longer periods of dryness (Schär et al. 2004). The developed method to directly measure *SA/V* ratios has the potential to better predict water loss rates than body mass allometry alone.

While physiological traits such as water loss are influenced mainly by varying environmental conditions, morphological traits could help to predict the vulnerability of species and communities to land-use intensification. Moreover, there can be traits like body size that were affected by both climatic variations (temperature) and land-use intensity.

Our findings highlight that different functional traits may be more sensible indicators of land-use intensity than species diversity alone. Functional traits are a promising possibility to assess the vulnerability of species and communities to land-use intensification and climate change. Specific processes are affected by several traits, while particular key traits can be simultaneously involved in the control of multiple processes (de Bello et al. 2010). Therefore, functional traits can link biodiversity responses of communities to the delivery of different ecosystem services (Diaz et al. 2007).

This should be also applied in conservation management, where up to today mostly only the diversity of species is used in the assessment of what species or habitats are endangered. Including functional components of communities in measuring biodiversity, instead of using the number of species, can help to reflect important structural properties of communities (Moretti et al. 2009). Functional traits as standard indicators can help to generalize monitoring practices across trophic levels and disciplines (Vandewalle et al. 2010). While a community of specific species cannot be easily extrapolated to different regions, traits can be generalized across functional groups and regions (Statzner et al. 2001; Hodgson et al. 2005). Therefore, the development of standard methods to measure traits and global databases are essential for all types of biodiversity monitoring (Green et al. 2005). A disadvantage of this method is that rare species or species with a particular conservation property are not considered explicitly as they are weighted by their low abundance. That can lead to false conclusions in management decisions. Functional traits should therefore, complement and not totally replace traditional biodiversity monitoring (Vandewalle et al. 2010).

Generally, the trait-based approach is suited for pollinators, as they are numerous and diverse and can be sampled relatively easily (e.g. passively with traps). Moreover, one can observe their activity patterns relatively easy (compared to e.g. herbivores or soils organisms in the field). Specific habitat or resource requirements make them responsive to environmental change (Steffan-Dewenter & Tscharntke 2000; Moretti & Legg 2009). Especially the measurement of morphological traits (e.g. body

size) does not require strong specific taxonomical knowledge or specific equipment and could, therefore, provide a less expensive solution for biodiversity monitoring. Nevertheless, one should focus not only on specific traits but also include morphological traits (chapter VI), physiological traits (chapter II and III) und life-history traits (Dziock 2006; Williams et al. 2010; Börschig 2012) to more fully represent the community responses to environmental changes and land-use intensification.

So far we only tried to estimate the consequences of already occurred disturbances to species, communities or ecosystems. Using the functional traits and response diversity in conservation management we can identify potential threads in future changes and take steps against them.

Potential topics for further studies

The response diversity of pollinator communities, determined in Chapter II, as well as the variation in traits means as an indicator for resilience in Chapter III, could also be assessed for other environmental factors for example wind speed, because there is not only one environmental factor that can affect pollinators. In the case of wind, not the diversity of responses to wind speed is important, but that there are as many pollinator species that cover even high wind speeds. All pollinators are able to forage when there is no wind. One also should quantify the reproductive success of the plants on the grassland sites to better predict effects of land-use intensity on the ecosystems. This process is labor-intensive, as seeds have to be counted and germination capacity has to be checked for many representative plant species. It should be noted that not all plants are obligate insect pollinated, but some can self-pollinate in case of the absence of pollinators. Nevertheless, it is assumed that pollinators can increase the reproductive success also for the self-compatible plant species (Steffan-Dewenter & Tscharntke 1999). It remains unknown whether the plants' reproductive success in intensively used areas is worse, despite the thermal resilience and the high species richness of pollinators. Whether flies that are dominant in intensively used grasslands are as effective pollinators as bees is controversially discussed (McGregor 1976; Watanabe 1994). The results of Chapter II and IV indicate no negative effects of land-use intensity on the resilience of ecosystems. One should keep in mind that land-use in our study did not include habitat loss, conversion or fragmentation. Pollinators are known to react very sensitive to such kinds of strong destruction (Winfree & Kremen 2009; Potts *et al.* 2010). Therefore carefulness is required before generalizing the results.

The method to create the 3D models of insects, used in Chapter III, should be further improved. In addition to turning the needled animals by 360°, the objects should be inclined to get better views

from above and below. If it is possible one could construct these models even with images from synchrotron μCT. Although these are very time consuming in processing until a completed 3D model is finished, they offer the possibility to measure a number of different traits. Also traits inside the body, like the size of different muscles could be measured with these models e.g. (Schmelzle *et al.* 2010).

Insects are able to adopt to dryness, as studies of insects in deserts show (Ahearn & Hadley 1969; Hadley 1970) and can reduce their water loss, for example by discontinuous gas exchange (Quinlan & Gibbs 2006). This potential to adapt to the environment should be better investigated – how fast can it develop, where are the limits – to make even better predictions of vulnerability to environmental variation.

One should also investigate the relationship between water loss of insects and cuticular waxes that covers the surface of the insects. Variations in the amount and composition of cuticle waxes could cause different water loss rates. Despite of the commonly known fact that the cuticle protects insects from water loss, relatively little in known about differences in cuticle thickness and composition across taxa and the implications for water loss (Gibbs & Pomonis 1995; Gibbs 1998). The wax layer can be removed by washing with a solvent (Wigglesworth 1945) and then analyzed with GCMS (gas chromatography mass spectrometry) to determine the composition and quantities of the substances.

Generally, one should also consider involving factors of the surrounding landscape to assess the impact of land use on pollinator communities. The measurement of land-use intensity included only the treatment that was applied directly to the grassland sites on which pollinators were observed. The proportion of semi-natural habitats that e.g. serve as nesting sites for pollinators, or the complexity or connectivity of the surrounding landscape was not considered. These factors are known to affect pollinator diversity and abundance (Steffan-Dewenter & Tscharntke 1999; Öckinger & Smith 2006). Also the proportion of mass-flowering crops like oilseed rape or sunflowers in the surrounding landscape can affect pollinators (Westphal, Steffan-Dewenter & Tscharntke 2003; Holzschuh *et al.* 2011).

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Ehrenwörtliche Erklärung

Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe.

Sämtliche aus fremden Quellen direkt oder indirekt übernommenen Gedanken sowie sämtliche von Anderen direkt oder indirekt übernommenen Daten, Techniken und Materialien sind als solche kenntlich gemacht. Die Arbeit wurde bisher bei keiner anderen Hochschule zu Prüfungszwecken eingereicht.

Darmstadt, den 31.08.2015

Curriculum vitae

Sara Kühsel

Date of birth:	09.07.1988
Place of birth:	in Dresden, Germany

Work Experience

05/2012 – 04/2015 — Scientific assistant at the Technische Universität Darmstadt
Working group: Ecological networks

- PhD-thesis in the context of the DFG-project „Biodiversity exploratories“, topic: “Pollinator trait diversity: functional implications at different land-use intensities and environmental conditions”
- mentoring of students (Bachelor-theses Master-theses, and Examination papers

Education

10/2009 – 04/2012 — Graduated studies in Biodiversity and Ecology at the University of Bayreuth

- major subjects: ecology, environmental sciences, zoology
- Master thesis at the University of Würzburg in the context oft he EU-project „STEP - Status and Trends of European Pollinators“, topic: Effects of oilseed rape on pollinators and the fruit set of wild plants”
- Master of Science, very good (1,1)

10/2006 – 09/2009 — Undergraduate studies in Biology at the University of Bayreuth

- major subjects: ecological and organismic biology
- Bachelor thesis in the context of the project „Biodiversity Exploratories“, topic: Effects of oilseed rape on the nesting behavior oft he red mason bee (*Osmia bicornis)*
- Bachelor of Science, good (2,2)

1998 – 2006 — Secondary school, Geschwister Scholl Gymnasium Nossen, Germany
Abitur (University entrance diploma)

Publications

Publications in peer-reviewed journals:

Kühsel, S; Blüthgen, N: Drivers of thermal resilience in highly diverse pollinator communities in managed grasslands, published in „*Nature Communications*“

Kühsel, S; Brückner, AK; Schmelzle, S; Heethoff, M; Blüthgen, N: Surface area - volume ratio in pollinating insects, under review in „Insect Science“

Kühsel, S; Büthgen, N: Land-use intensity as a filter of morphological trait composition: pollinator communities in meadows and pastures, in preparation

Conference contributions:

Kühsel, S (2014): Pollinator responses to temperature and the effects of land use. Talk at the 44th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland.

Kühsel, S (2013): Effects of different weather conditions on pollinator activity and body temperatures. Poster at the 10th Annual Meeting of the German Biodiversity Exploratories.

Additional skills

Languages:	German (first language), English (good skills), (Russian, French, Spanish (knowledge acquired at school)
Software:	Office Word, Excel including Pivot tables, Power Point, Access (proficiency) multivariate statistics with R (proficiency) Arc GIS (basic knowledge) research and reference manager Endnote (proficiency)
Internship:	6-week practical training (2008) at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Sylt; Topics: ecological processes in the biotope Wadden Sea and adjacent North Sea, benthos monitoring with the research vessel „Heincke“

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